BUREAU OF HYGIENE AND TROPICAL DISEASES

BULLETIN OF HYGIENE

Volume 36, No. 11 November, 1961

Public Health

The order in this section is:—**General; Vital Statistics; Epidemiology; Maternal and Child Health; Health of Students, Nurses and Hospital Staffs; Health of Old Persons and the Chronically Ill; Chronic Cardio-Respiratory Diseases; Chronic Rheumatism; Cancer; Miscellaneous Diseases; Dental Health; Mental Health.**

GENERAL

MacQueen, I. A. G. **The Future of Public Health.** *Med. Officer.* 1961, July 7, v. 106, No. 1, 5–8.

This contribution by the Medical Officer of Health of Aberdeen is part of the Education Number published annually by the *Medical Officer.*

In the "hungry '30s" the work of a public health officer was essentially medical and he could have personal knowledge of the whole field of public health; now there is a multi-professional team and it must be recognized that some members of it such as the superintendent health visitor, the health education officer and the statistician possess professional skills that the doctor lacks.

In the last 2 or 3 decades there have been two trends. (a) declining importance: a gradual but steady decrease in the problems of environmental hygiene, infectious disease control and personal health services for the physical health of children.

(b) increasing importance: growing stress on the promotion of mental health; prevention of maladjustment, delinquency and neurosis; reduction of home and road accidents; maintenance of the health of the aged; research into diseases of maturity; and provision of supportive services for the frail and the handicapped. Unless the public health service accepts the challenges of (b) other people will take them over. In the prevention of mental ill-health the family health visitor can play an important part: accidents can be prevented by education about how they occur. On the care of the aged the author presses for the amalgamation of the Health and the Welfare Departments and the avoidance of duplicate home visiting by officers of both.

The paper includes a strong plea for enhancing the status and pay of health visitors with animadversions on medical officers of health for their failure to give active support to the claims of health visitors for better conditions of service. The warning is given that prospective health visitors may take up training in social work and obtain more pay in Welfare or Children's Departments.

The dangers confronting health departments result from (1) obsession with the clinical side; (2) inability to recognize that non-medical staff have skills that doctors do not possess and (3) lack of interest in the well-being of non-medical staff. It must be remembered that "outside the Health Department are a horde of empire-builders ready to seize any tasks that we neglect and ready to fight for good salaries and conditions for members of their teams".

The author, however, concludes that he believes "that the greatest days of the public health service lie ahead".

[This is a forthright attack on complacency and apathy in the higher ranks of the public health service and should be read by all who have the future of the service at heart.] *Allen Daley*

London School Hyg. & Trop. Med. **The Views of General Practitioners, Aldermen, Councillors, and Teachers on Some Aspects of Public Health. By a Study Group in the Public Health Dept.** *Med. Officer.* 1961, July 7, v. 106, No. 1, 9–16.

This is an exercise in fact-finding and report-preparation undertaken early in 1961 by a group of students taking the DPH course at the London School of Hygiene and Tropical Medicine.

A questionnaire containing 21 questions was drafted: it appears in full in the appendix. The same questionnaire was used for all 3 groups: general practitioners, members of local authorities and school teachers. The questions were designed to ascertain opinion about selected facets of public health work including present activities, what developments there should be, and which of a number of specified possibles has contributed most to recent reductions in infant mortality.

The money available was small and a selection had to be made of those to whom the questionnaire might be sent. The local authority areas chosen for the enquiry were that of the county of London and of the urban district of Bishop's Stortford, Hertfordshire. The population of the former is 3,204,000 and of the latter 17,240.

In Bishop's Stortford all the doctors, all the members of the local council and all the teachers in the 2 secondary modern schools were invited to complete the questionnaire; in London random sampling had to be

used, namely, 1 in 4 of the 146 members of the County Council and 1 in 33 of the general practitioners on the list of the London Executive Council. The doctors and the councillors received the questionnaires by post: the teachers got them from the head teachers who had been visited by a member of the study group. 54 out of the 68 London doctors approached and 10 of the 11 Bishop's Stortford doctors filled them in as did 27 of the 36 members of the London County Council and 13 of the 16 Bishop's Stortford members: all 82 teachers completed the questionnaire, 48 in London and 34 in Bishop's Stortford. A few of the answers from the doctors were facetious or offensive but most had given careful thought to them.

The paper itself must be consulted for details of the replies to the separate questions.

In brief, 26 of the 52 London doctors thought that a school medical officer was still necessary as did 8 of the 10 in Bishop's Stortford; and 35 of the 48 London teachers and 33 of those in Bishop's Stortford out of 34 agreed with them. 52% of the doctors, 88% of the councillors and 80% of the teachers thought that the public should be made more aware of the early signs and symptoms of cancer. A separate question asking if any had first-hand evidence of ill-effects of health education resulted in 21% of the doctors answering "yes" and 77% "no". 66% of the doctors, 44% of the councillors and 38% of the teachers were in favour of the fluoridation of water. Substantial majorities in all 3 groups thought that the services now administered by the County MOH should continue to be so administered. A question enquiring if the last contact with an officer of the local health authority had been satisfactory resulted in 92% affirmative replies from the doctors, 97% from the councillors and 81% from the teachers.

It is concluded that the enquiry had been useful though it is admitted that it is only in the nature of a pilot study.

[Also, it would have been better to have had a separate questionnaire for each of the 3 groups and excluded questions about which the recipients would not be expected to know the answers. For example the question "What in your opinion is the most common cause of sickness absence in industry today?" is quite suitable for doctors but not for teachers and not for most councillors.] *Allen Daley*

BROCKINGTON, C. F. **Training for Public Health and Medical Administration.** *Med. Officer.* 1961, July 7, v. 106, No. 1, 17–20.

The General Medical Council sets the standard for the Diploma in Public Health which in Great Britain is a statutory requirement for those appointed as medical officer of health of counties, boroughs and urban and rural districts.

The standard is designed to ensure that its holders have attained "a distinctively high standard of proficiency, scientific and practical, in all branches of study which concern the Public Health".

The current rules were adopted in November 1955 and superseded those of 10 years earlier. Revision had become essential owing to the changes in the work of medical officers of health brought about by the Nationa Health Service which came into operation in July 194

The main changes in the 1955 rules were (1) registered medical practitioner could start his DPH cours immediately after full registration instead of having t wait 2 years (in the meantime there had been a require ment that a year's appointment as a house officer in a approved hospital must have been completed before ful registration). (2) in the 1945 rules a preliminary cours leading to the award of a certificate in public healt (CPH) had been required before taking the final course In 1955 this was abolished and there is now a singl unified course for the DPH and no more examination for the CPH will be held.

The present course for the diploma covers either a academic year's whole-time study or an equivalent cours of part-time study lasting 18 months or a combinatio of whole and part-time of not less than 15 months. An employment undertaken during a period of part-tim study must be approved by the teaching body concerned Rule 2, set out in full in the paper, prescribes the sub jects to be covered in the theoretical instruction. Rule retains, in a modified form, the requirement that th candidate shall receive instruction in a hospital fo infectious diseases and Rule 4 requires attendance at a approved health department during a period of not les than 5 months. The candidate must keep and present t the examiners a day book recording the subjects in whic he has been instructed as well as a dissertation on subject approved by his teaching school.

After setting out the above the paper discusses trainin for medical administration and quotes the dilemma pose by the President of the Royal College of Physicians o London in 1953 namely "whether its members (*i.e.*, o every profession) shall concentrate on 'strictly profes sional' work and lose their power to direct it, or lear administration so as to be able to remain in control o it, thus losing the time to practise it". Assuming tha medical administrators will continue to be needed th possibilities are that one or more universities will provid a course of study leading to a Diploma in Medica Administration; or the DPH course might be adapted t become an overall qualification suitable for any typ of medical administration. The author favours the latte on the grounds that medical administration needs to b based on (1) knowledge of the government of the healt services, (2) understanding of human behaviour an motivation, and (3) the epidemiology of health an disease, all three of which are equally important for th DPH student. *Allen Daley*

WOFINDEN, R. C. **The Changing Pattern of Medic Social Service in the Home in Bristol.** *Pu Health.* 1961, July, v. 75, No. 5, 304–11.

In 1954 the author published the results of an enqui made in Bristol in 1951 into family visiting [this *Bulleti* 1954, v. 29, 705]: at that time there were 35 differe kinds of socio-medical workers who might have visited family: 964 families were included in the survey. Th conclusion was "that there was no undue multiplicity visiting by different organisations" and that even wh

4 or more had visited the same family in the previous 5 months there had been a good reason for it.

The enquiry was repeated in June 1958. In the meantime the report of the Working Party on Health Visitors had recommended in 1956 that the health visitor should be regarded as the primary medico-social worker in the home; the welfare department had extended its services for the aged and handicapped; new voluntary organizations had been set up and old ones had extended their activities; and care committees to co-ordinate the visits of field workers had been established.

In 1958 there were 17 different types of visitors in the health department; 7 in other departments of the local authority; 2 from hospitals; 3 from central government departments; and 17 from voluntary organizations which together with doctors and clergy made a total of 51. The enquiry which lasted a week and embraced 1,227 households was made by health visitors who in the course of their visits enquired what other visitors from the list had been there in the preceding 6 months. The family doctor had visited 868 (nearly 71%) including 90, 81%, of the 111 problem families and 104, nearly 90%, of the 117 sick or aged: 14·4% of the total had been visited from the church or chapel.

Tables show how many households had received multiple visits. In 1951, 2·7% had had more than 4 visitors but in 1958 this had risen to 5·0%; this figure, however, includes only visitors from the 1951 list of 35 possible visitors, but if the additions made in the 1958 list of 51 possibles, *i.e.*, 16 more, are included the percentage of households which had had more than 4 visitors becomes 13·24.

In discussing the reasons for this increase the author mentions that statutory officers cannot delegate statutory duties to a designated co-ordinating officer nominated by a co-ordinating committee and points out that the "increased coverage" is provided mainly by visitors employed by the health department and the National Assistance Board rather than by other agencies statutory or voluntary.

The main objective of case co-ordinating committees which was to minimize multiple visiting has not been achieved but they have ensured a more co-ordinated approach to a family's problems.

The percentage of households receiving no visit, other than that of the health visitor, during the previous 6 months was smaller in 1958 than in 1951 which indicates that visiting is now more selective. *Allen Daley*

SALUD PÚBLICA MÉXICO. 1961, Jan.–Feb., v. 3, No. 1, 9–123, illustrated. [25 refs.] Primer Congreso Mexicano de Salud Pública. **[First Mexican Congress on Public Health]**

The first Mexican Congress on Public Health was held in December, 1960, to review progress in public health and social welfare since the beginning of the revolution in 1910. A selection of the papers presented at the Congress is published.

The first paper, by J. A. AMEZQUITA (pp. 9–14) gives some statistics to show the improvements during this century in the control of major diseases and the reduction in mortality, particularly of infants (before 1900, estimated at 335 per 1,000 live birth; in 1930, 131·6 per 1,000; in 1959, 75·5 per 1,000). Births rose from 495,500 n 1900 to 1,565,000 in 1959. C. ZUCKERMANN (pp. 15–18) explains the organization of the departments dealing with medical services, social welfare, maternal and infant services, rehabilitation and mental illness; M. E. BUSTAMANTE (pp. 19–60) describes the principles guiding the work of these services and lists the main types of medical institutions. He also considers the problem, common to many countries, of attracting entrants to medicine in competition with general science and engineering. A table shows the populations in various countries in 1957 served by one doctor—*e.g.*, 952 in Canada, 781 in U.S.A. and 1,740 in Mexico. The effects of natural conditions, social structure and epidemiology on the organization of the health services are discussed in a paper by X. de la RIVA R., (pp. 61–66), and the functions, programme and organization of the Registro Nacional de Anatomia Patológica (founded in 1959) are described by G. A. FUERTES (pp. 79–84).

Further contributions on a wider geographical basis include an outline of the part played by international organizations in public health programmes (M. G. CANDAU, pp. 67–72), economic aspects of health (A. HORWITZ, pp. 73–78), mental health and social evolution (M. M. V. SUAREZ and H. M. CABILDO, pp. 85–89) and social well-being in rural communities (J. F. ORTIZ, F. N. CHAVEZ and A. A. ORTIZ, pp. 91–97). *A. H. Phipps*

VITAL STATISTICS

WYNNE-GRIFFITH, G. & MORGAN, G. A. V. **Diagnostic Precision as a Factor in Male Mortality Data.** *Brit. J. Preventive & Social Med.* 1961, Apr., v. 15, No. 2, 68–78, 2 figs. [20 refs.]

The mortality experience of males in England and Wales during 1950–53 has been examined by the method of factor analysis. The six administrative areas with a population of less than 40,000 were discarded, leaving 167 areas for the analysis. The components used were rates from 22 causes of death and 17 indices of socio-economic status. The findings of this analysis were discussed and compared with the results obtained by other workers using the more conventional methods of comparison. For several causes of death there appears to be a factor in the level of mortality which represents diagnostic precision. Some of the variation between administrative areas for certain causes of death can be interpreted as variations in diagnosis. *W. J. Martin*

EPIDEMIOLOGY

GRAFFAR, M., CORBISIÈR, J., VAN DEN BERGHE, F. & ASIEL, Monique. Étude comparée de l'état sanitaire et social des enfants dans une région urbaine et dans une région rurale. **[Comparative Study of the Socio-economic State and Hygienic Environment of Children in an Urban and a Rural Area]** *Arch. Belges Méd. Sociale, Hyg., Méd. du Travail et Méd. Légale.* 1961, Feb., v. 19, No. 2, 77–124, 17 graphs.

This paper contains the first results of an official investigation in a rural area and an urban district in Belgium to ascertain and compare the social state and hygienic environment of children living in these districts. The study dealt with 338 families with 339 infants and 482 older children in the rural area and 203 families with 224 infants and 123 other children in the urban areas.

The findings of the study are described in considerable detail with graphs and tables in chapters on the demography of the areas, the composition of the families, the level of education of the parents, the sources of income and type of employment of the heads of the families, the housing conditions and the distribution o different socio-economic levels.

As the level of education of the parents and hence th economic status in the urban was higher than in the rura area, the other differences were not remarkable o unexpected. Housing conditions were often unsatisfac tory and overcrowding was present in both areas. I certain respects such as water supply and genera sanitation the rural families were worse off than th urban. *M. E. Delafield*

PEMBERTON, J. & WILLARD, H. **Recent Studies i Epidemiology.**

This book was reviewed on p. 958.

MACMAHON, B., PUGH, T. F. & IPSEN, J. **Epidemiologi Methods.**

This book was reviewed on p. 957.

MATERNAL AND CHILD HEALTH

DONABEDIAN, A. & ROSENFELD, L. S. **Some Factors influencing Prenatal Care.** *New England J. of Med.* 1961, July 6, v. 265, No. 1, 1–6. [11 refs.]

This article reports the results of a survey of pre-natal care made in June 1956 in the Boston Metropolitan area. A comprehensive questionnaire was given on the 2nd or 3rd post-partum day to all mothers in a selected group of hospitals during a period of one month.

A standard of adequacy of care was agreed and it was found that about one-third had not received it and that nearly two-thirds had had no dental care during pregnancy. The percentage receiving adequate care rose from 37·9 in the lowest to 93·2 in the highest income group and from 37 among those with the least to 83·6 among those with the most general education. The findings "suggest that higher education can, in some degree, offset the handicap imposed by low income upon the receipt of adequate pre-natal care" but the warning is sounded that the population of the group studied had a somewhat narrow range of income (49% between $3,000 and $4,000 a year) and of education (48% had completed high school).

In the opinion of 83% of those who completed the questionnaire pre-natal care should begin during the first trimester of pregnancy and a table shows the relativity between when the various groups thought that it should begin and when, in fact, they sought it. Fewer than 70·4% obtained it within the first trimester compared with 82·6% who thought that it should be initiated then: the discrepancy between opinion and performance is greatest in the lower income and poorer education groups. With increasing parity fewer think that it i necessary to start pre-natal care early or actually obtai it. The amount of care provided was regarded as satis factory in 82% of those who obtained it from a privat doctor but only in 41% of those who went to a publi clinic. It is not known whether this difference is du to the kinds of people who chose these forms of care o to the different standards of those providing the service The content of the care was investigated and it was foun that 6·4% had never had a pelvic examination: for th 1-para it was 2·4%; for the 2-para, 6·7%; for the 3- an 4-para, 8·3% and for the 5- or more para, 11·7%.

General maternal instruction in such things as die rest and exercise, clothing and "danger signs" was rate as "good" in 62%, "fair" in 14% and "poor" in 24%

The conclusion is that further efforts are needed t convince mothers of poor education, those in the lowe income groups and those who have already had sever children that advantage should be taken of facilities fo pre-natal care from early in pregnancy. *Allen Daley*

MINISTRY OF HEALTH. CENTRAL HEALTH SERVICE COUNCIL. **Report of the Sub-Committee on th Prevention of Prematurity and the Care of Pr mature Infants** [CLAYE, A., Chairman]. 8 p 1961. London: H.M. Stationery Office. [9d.]

The committee which prepared this report contain 13 members of whom 3 were midwives and the re medical.

Prematurity is defined internationally by birth weight (2,500 gm. or less) and not by the estimated period of gestation. 58,648 babies were born prematurely in England and Wales in 1959: this was 6·7% of all notified live births; also in that year 54% of the 15,403 stillbirths were premature. In about 50% of premature births the cause is unknown. Further research is needed to establish the aetiology of this condition. The most important associated conditions are toxaemia, ante-partum haemorrhage and multiple pregnancy. The main handicaps of those born prematurely are inability of the lungs to function, increased risk of cerebral birth trauma and intracranial haemorrhage, immaturity of the liver and the digestive tract, poor heat-regulating mechanism and susceptibility to infection.

The rate of survival is directly proportional to the birth weight (only 34% of those under 1,500 gm. survive). The first day of life is the most dangerous (46% of those under 1,500 gm. birth weight die within 24 hours). "The prognosis for premature infants in the higher weight groups is excellent" and though 8% of all those born prematurely by the weight definition die within 24 hours, 86% survive until at least 28 days.

Enquiry was made from the 146 local health authorities and from 377 hospitals in England and Wales about the facilities available for hospital or home care of the premature: they vary widely and only 45% of those in the lowest weight group, under 1,500 gm., were admitted to specially equipped premature baby wards. A description is given of a comprehensive programme for premature infants. It comprises measures to reduce the incidence, an integrated scheme to cover all aspects of hospital and home care and an effective system of after-care. For prevention high quality ante-natal supervision is important and admission to hospital whenever indicated. Special baby care units should admit mature sick newborn infants as well as the prematures. Such units should be provided only in large or medium-sized maternity departments catering for abnormal obstetrics. For every 1,000 births at least 6 cots for prematures and other sick newborn infants are needed; the average stay is about 5 weeks; the optimum size is not less than 20 cots, which should be adequate for a population of 200,000. 30 sq. ft. of floor space are needed for each closed incubator and 50 for each cot or opening incubator. There should not be more than 6 cots or incubators in any room. A consultant paediatrician, associated with a consultant obstetrician, should be in charge and the nursing staff should number 2 for every 3 babies; half the nursing staff should have been specially trained.

An emergency resuscitation team from the unit should be available to proceed at a moment's notice to a small maternity hospital or any home where there is a premature baby. All women in premature labour should, if time permits, be admitted to the area specialist centre. The percentage of oxygen in incubators must be strictly controlled—25% is normally adequate. The premature infant must be protected carefully from infection and barrier nursing practised.

In 1959, of all notified premature births, 22% occurred at home and 20% of the live-born premature infants born at home were transferred to hospital. This is far too many home premature confinements but there will always be some and provision must be made for them. These must include facilities for immediate resuscitation after which many will need admission forthwith to a special unit. The journey must be in a heated ambulance with a heated carrier with provision for oxygen administration. A specially trained nurse must travel with the child. For those who remain at home and for the after-care of those discharged from hospital special equipment is needed and specially trained nurses or midwives with a small case load as daily visits may be necessary for 6 to 8 weeks and weekly thereafter for some time.

A leaflet should be prepared for general practitioners and all others concerned setting out the local arrangements for dealing with premature infants and stating exactly how they can be brought into action when needed. *Allen Daley*

HEALTH OF OLD PERSONS AND THE CHRONICALLY ILL

ENGBERG, Eugenie. **Family Flats with a Nursing Annexe. A Danish Experiment for the Disabled.** *Lancet.* 1961, May 20, 1106–7.

This is an interesting short account of a new project being carried out in Copenhagen to provide flats for severely disabled persons where they can live with their families but at the same time have available in the same building the help of an institution.

In Denmark a considerable number of persons survived the poliomyelitis epidemic of 1952 with persisting respiratory paralysis. These have been in hospital for 8 years with resulting disruption of family life for adults and for children. Some of these disabled persons and indeed persons disabled from other causes might be restored to family life provided nursing and medical care were readily available.

The project described here is housed in a new block of 170 flats of which 14 are reserved for respiratory patients and their families. Each of these 14 flats has extra electrical equipment and safety installations. On the top floor of the building there is a nursing annexe with professional staff and emergency equipment. It has cubicles where patients can sleep under professional supervision at night when they are ill or when their families are on holiday. The annexe has living rooms, a classroom for education and entertainment and a roof garden.

Experience has shown that a member of the family is able after some training and with some help from the nurses and doctors of the annexe to look after the respiratory patient. Such a member of the family is employed by and paid by the Government to take on this responsibility.

The rent of these 14 flats is paid by a semi-public organization representing the Ministry of Social Affairs, the National Foundation against Poliomyelitis and the Society and Home for the Crippled.

It is much too early to evaluate the results of the project but some preliminary conclusions are:—(1) severely handicapped persons and their families have been enabled to function as a family unit, (2) badly needed hospital beds and personnel have been released for other purposes, and (3) public funds have been saved, since the cost of maintaining a patient in his own home is less by one-third than the cost of maintaining him in hospital. *M. E. Delafield*

BRESARD, M. Enquête sur les conditions de vie des personnes agées. I. Les données sociologiques. [**Survey of Living Conditions in Old Persons. I. Sociological Data.**] *Bull. Inst. Nat. Hyg.* Paris. 1961, Jan.–Feb., v. 16, No. 1, 65–126, 30 graphs. II. Les données alimentaires. [**II. Dietetic Data**] *Ibid.*, Mar.–Apr., No. 2, 305–33, 16 graphs.

In prosperous countries with highly developed health services the percentage of old people in the population is rising year by year. As a result, increasing attention is being given, in most of these countries, to social and health problems associated with old age. The publications reviewed here deal with groups of men and women aged 70 and over living in Saint-Etienne and Marseilles in France. Part I is essentially a study of social and health conditions, while Part II is concerned with diet. The information was obtained by interviews and questionnaires, accepted without enthusiasm by the old people, whose French individualism was unaffected by advancing years.

It cannot be said that anything new or unexpected emerged from the investigation. Most of the men who had retired prematurely (at 62 or under) regretted it, but this attitude was not found in those remaining at work until 66 or over. The earlier the retirement, the lower the standard of living. In general, the healthiest subjects were those with long-lived parents. Complex interrelationships were elicited between physical activity, the possession of satisfactory dentures, smoking, residence in a given apartment storey with or without a lift, and other physical and environmental factors. One fact was clearly evident, namely that people still living in a family circle withstood the process of ageing more successfully than those deprived of contact with descendants and family life. The same observation, of considerable sociological significance, has been made in other countries.

The calorie intake of the majority was sufficient, given their reduced energy expenditure. 24% of the men consumed as much food as active young adults; at the other end of the scale were 10% existing on the average on 1,540 calories daily. The men ate more meat but less vegetables and fruits than the women, and also drank more wine. There were no obvious qualitative defects in the diets consumed.

[No doubt it is useful to have facts of this nature, but they throw little light on the special dietary requirements of old people, if indeed old people have special dietary requirements. No serious attempts have as yet been made to study this problem anywhere, so that little or nothing is known about the relation between diet and health in old age.] *W. R. Aykroyd*

CHRONIC CARDIO-RESPIRATORY DISEASES

SCHROEDER, H.A.; DURÁN, J. **Hardness of Local Water-Supplies and Mortality from Cardiovascular Disease.** [Correspondence.] *Lancet.* 1961, May 27, 1171.

The results of MORRIS *et al.* [this *Bulletin*, 1961, v. 36, 729] are very similar to those found in the U.S.A. by Schroeder [*ibid.*, 1960, v. 35, 1159]. But, Schroeder now writes, in Japan there are also large regional differences in mortality from vascular disease of the central nervous system and lesser ones from heart diseases and these do not correlate with the hardness of water or with any common constituent except the sulphate-bicarbonate ratios. If the same factor influencing death rates in the U.S. and England is operating in Japan, it is not necessarily associated with hardness of water but can also act through soft waters of varying degrees of acidity. There are other unexplained differences in the results for the three countries, *e.g.*, in the U.S. the highest correlations were found with hypertensive heart disease; these were fairly low in England. The lowest significant correlations were with cerebrovascular disease and these were high in England and Japan. Whatever the "water factor" may be it certainly cannot at present be considered as causal.

Dr. Durán points out that in the Canary Islands the incidence of cerebrovascular and coronary heart disease is very high, animal fat in the diet is negligible and blood-cholesterol levels unusually low, but the drinking water is exceedingly soft. *Austin Bradford Hill*

WISE, R. A., ENGELHARDT, H. T., SNYDER, H. B. & BAIRD, V. C. **Coronary Artery Disease II: Age-Specific Incidence of First Myocardial Infarction in White Males.** *Amer. J. Pub. Health* 1961, June, v. 51, No. 6, 866–72, 2 figs.

13,900 white men and 2,400 white women aged 30–6 working for a petroleum company were studied durin the years 1954–57. These individuals performed a varieties of labour, from desk work to manual, and ha varying degrees of responsibility. Notification of th occurrence of a myocardial infarct was obtained fro "proof of sickness" (as all employees receive full pa during sickness) and study of the electro-cardiogra which was nearly always taken in such a case, was mad

A total of 222 first myocardial infarctions occurred in the men. Age-specific attack rates have been calculated for each year. These range from 0·0·40 per 1,000 employees per annum at 30–34 years to 8·0–11·16 per 1,000 employees per annum at 55–59 years. No infarct occurred in the women workers, but most were under 40 years old.
W. W. Holland

DUTREY, M. Le traitement des bronchites chroniques par un complexe à la prednisone. **[Treatment of Chronic Bronchitis by a Complex including Prednisone]** *Bruxelles-Méd.* 1961, Feb. 26, v. 41, No. 9, 281–5. [13 refs.]

This is a review, dogmatic in type, of the aetiological factors in chronic bronchitis, together with a recommended palliative polypharmaceutical treatment designed to cope with the various pathological phenomena commonly found in the chest—bronchial oedema, spasm, and hypersecretion of mucus, all conspiring to produce bronchial obstruction and the resultant cor pulmonale. Tablets containing the following are to be taken 3 or 4 times a day: prednisone 1·5 mgm., theophylline 80 mgm., ephedrine 10 mgm., and phenobarbitone 10 mgm. [It is all rather theoretical and there is no statistical or other account of the cases treated.] *H. Stanley Banks*

See also p. 1105, HIGGINS, **Bronchitis.**

CHRONIC RHEUMATISM

STEINBERG, S. S. & FITZPATRICK, E. D. **The Paducah Health Education Survey, 1958.** *Amer. J. Pub. Health.* 1961, May, v. 51, No. 5, 732–45.

A study was undertaken in Paducah, Kentucky, U.S.A. (population about 47,000) in 1958 to evaluate certain aspects of a health information campaign about the problems of arthritis and rheumatism. The study was conducted in 4 stages. First a sample of the population (selected randomly from household addresses in the city directory) was interviewed by students using a standard questionnaire. The results of this gave an idea of the level of knowledge existing before the campaign. For three days immediately following the interviews, press, radio and television carried many articles, features and spot announcements about different aspects of the problems. On the second day following the completion of the intensive information campaign the original sample was re-tested and an additional group of the population (control group) was tested. The fourth and final stage consisted of a further test of the original and control samples after a lapse of 6 months to see how much knowledge about arthritis and rheumatism had been retained.

The previous educational achievements of the people interviewed were noted and it was found that those who had had secondary and college education had acquired more information from the campaign than those who had only had an elementary education. The most interesting findings, however, were that the group who had been interviewed before the campaign had learned more from the campaign than the control group; and that 6 months later both groups had a similar level of knowledge, which was higher than it had been at the time of the first interviews of each group. Therefore the control group had acquired more knowledge about the conditions after the intensive campaign had ceased. The authors conclude that the lay public can be "sensitized" or "motivated" to seek information about a disease by personal contact of the questionnaire-interview type, and that this type of contact makes people more receptive of information provided in a deliberate campaign.
M. D. Warren

CANCER

LEGUÉRINAIS, J., LAURENT, C. & MAUJOL, L. Présentation des cas de cancer observés en 1958 dans les Républiques Africaines d'expression française. **[Incidence of Cancer in 1958 in the African Republics, formerly French]** *Bull. Inst. Nat. Hyg.* Paris. 1961, May–June, v. 16, No. 3, 521–31, 1 fig.

This is an attempt to carry on the annual cancer survey in the former French possessions in Africa. The returns are less complete than formerly and the recorded cases show a considerable fall in number. In the West African republics 551 cases of cancer were reported, of which one-third were confirmed by histological examination, and in the Equatorial republics 447 cases with almost one-half verified by histological examination were recorded. In the West African countries almost one-half of the male cases were of cancer of the liver, and in the Equatorial countries skin was the numerically most important male site, accounting for one-sixth of the cases. In both groups of countries the most important female site was the uterus. *W. J. Martin*

WIGLEY, S. C. & RANDMAE, G. **Lung Cancer and the Melanesian.** *Papua & New Guinea Med. J.* Port Moresby. 1961, Mar., v. 5, No. 1, 5–10, 20 figs. on 10 pls.

To draw attention to the fact that cancer of the lung occurs among the indigenous people of Papua and New Guinea, the authors briefly describe 28 undoubted cases that they have encountered in Melanesians, these being the residue of a larger number in which a strong presumptive diagnosis could not be further substantiated. While presenting no epidemiological evidence they note that all had smoked and inhaled deeply from an early age. *Austin Bradford Hill*

WYNDER, E. L. **An Appraisal of the Smoking-Lung-Cancer Issue.** *New England J. of Med.* 1961, June 15, v. 264, No. 24, 1235–40, 3 figs. [24 refs.]

This article by Dr. Wynder, of the Section of Epidemiology of the Sloan-Kettering Institute, and the rejoinder by Dr. LITTLE, Scientific Director of the Tobacco Industry Research Committee [see below], were presented at a meeting in Boston of the New England States Chapter of the American College of Chest Physicians. In arguing that cigarette smoking is a causative factor of cancer of the lung Dr. Wynder starts with presumptive evidence—that there has been a real increase in incidence of lung cancer over and above that due to improved diagnosis; that this increase has been primarily in men and not in women which makes it difficult to give a major role to air pollution; that it is known that heavy smoking may lead to chronic cough so that the suspected factor comes into immediate contact with the tissue where cancer develops; and that it is known that carcinogenic polycyclic hydrocarbons can be produced by burning organic matter such as tobacco. It is therefore no matter for surprise that the statistical and epidemiological data bear out this presumptive evidence. There have now been more than 25 retrospective studies conducted in 9 countries, and totalling more than 6,000 patients with lung cancer, as well as 3 prospective studies, and there is not one of them that does not show that patients with lung cancer smoke considerably more than control patients. The differences between smoker and non-smoker are large and any statistical bias or error to account for them would have to be of a similar large magnitude. None has been established. Similarly there is no evidence of the existence of constitutional factors of such magnitude as to account for the very different risks. Furthermore, such a constitutional factor would have to have increased in recent years, be sex-linked and absent in countries where lung cancer is uncommon. The observations of EASTCOTT [this *Bulletin*, 1956, v. 31, 405] and DEAN [*ibid.*, 1960, v. 35, 217] that British immigrants have more lung cancer than persons born in New Zealand or South Africa may suggest air pollution as an additional factor but the difference between the immigrants and the home populations are far smaller than those found between non-smokers and heavy smokers in whom lung cancer develops. The present sex ratio of lung cancer is consistent with the long-term smoking habits of the two sexes.

Various authors have contributed pathological evidence to the effect that there is an increase in metaplasia, hyperplasia and carcinoma *in situ* with increasing number of cigarettes smoked. Biological evidence shows that cigarette smoke condensate can be carcinogenic to mouse and rabbit epidermis and to the hilus of rats. Exposure of laboratory animals to cigarette smoke has produced no bronchogenic carcinoma but neither have experiments with benzpyrene mist. Unless one can find an animal that will inhale cigarettes as man does, it may be impossible to duplicate the human findings. But neither positive nor negative animal experiments can offer conclusive proof that man will react similarly. Chemical studies suggest that a chemical explanation of the biological findings may lie in the polycyclic hydrocarbons being initiating carcinogens and some of the phenols being powerful promoting substances.

The total sum of evidence, therefore, includes chemical evidence that explains at least in part the positive biological findings and pathological evidence that is in line with the statistical and epidemiological evidence, and all these evidences are in agreement with the presumptive evidence. The mechanism of carcinogenesis is unknown but one need not know the precise mechanism of causation to prevent a disease. How a given factor increases the risk may be relatively unimportant from the preventive aspect so long as one knows that in its absence the disease will occur less frequently. Finally, "if one criticizes epidemiology for being statistical, if one criticizes animal research for being unrelated to the human problem and if one criticizes chemical identification of carcinogens as not having any bearing to the human disease, I should like to ask if there is a form of evidence that would be accepted as being conclusive". *Austin Bradford Hill*

LITTLE, C. C. **Some Phases of the Problem of Smoking and Lung Cancer.** *New England J. of Med.* 1961, June 15, v. 264, No. 24, 1241–5.

Dr. Little's purpose is "to present certain of the reasons why all such data (which are at times based on rather superficial, scattered and indirect observations) fail to convince other scientists and medical men that cause-and-effect relation between tobacco use and bronchogenic carcinoma has been established.

"This does not mean that certain existing studies have not produced suggestive findings. It merely proposes that the many problems raised by such studies are not yet solved. . . ." To ask, as does Dr. WYNDER, what specific evidence would "convince" the unconvinced is unrealistic. If one could define such specific evidence the problem would be already solved.

From the pathological aspect areas of epithelial metaplasia in the lungs have been interpreted by some pathologists as being pre-cancerous areas associated with smoking. However, similar lesions have been found in infants, children and non-smokers so that the origin of such lesions may be independent of smoking. Such lesions are also found in the trachea and if smoking causes lung cancer why is it that the trachea does not form cancer as the lung does? An increase in these lesions has also been shown in persons afflicted with

pneumonia so that such an increase is not distinctly diagnostic of any effect of tobacco.

Under animal experimentation the more applicable research has been the exposure of the lung, not the skin, to whole smoke, not to a machine-made concentrate. No such experiment has produced carcinoma of the lung in animals. The results of the animal skin-painting work have been derived by use of concentrated chemical materials obtained from machine smoking that differ radically from those produced by human smoking and in which no substance known to be carcinogenic to man has been demonstrated.

Statistical evidence alone cannot be considered final or conclusive in establishing causation though it is here "suggestive and challenging". Weaknesses lie in the following features. There is no sure evidence as to how much, if any, of the reported increase of lung cancer mortality is real. Causes of death that have been related to excessive cigarette smoking in statistical studies include cancer of various sites, respiratory diseases, circulatory diseases, peptic ulcer and cirrhosis of the liver. Other medical literature includes eye trouble, nose and ear ailments, miscarriages and so on. It is not possible to accept such a wide range of effects and this indeed is a major area of disagreement among the statisticians themselves. Another area of disagreement lies in the question of inhaling. Some investigators in the U.S.A. believe that there is a greater proportional incidence of lung cancer among inhalers though they have produced no firm data in support. On the other hand, British scientists in a survey of doctors found virtually no difference in lung cancer incidence between inhalers and non-inhalers. [This is a serious misquotation in such a serious setting. DOLL and HILL (this *Bulletin*, 1954, v. 29, 1059; 1957, v. 32, 145, 557) in fact, have made no report whatever on the inhaling habits of doctors. Their only evidence on this point lay in their early retrospective inquiries into patients with lung cancer and controls. It is to this that Dr. Little is really referring and which, in his more extensive comments, he has erroneously translated to the prospective inquiry into doctors.]

The variation in the claimed "excess risk" of lung cancer among cigarette smokers is another example of conflicting opinion, as is also the wide difference of opinion concerning the relative quantitative role of cigarette smoking in the aetiology of lung cancer. The difference between the sexes has been widening rather than narrowing in recent years. There are differences in defining heavy, moderate and light smoking. Much of it is only an approximate estimate of past and present habits and not a scientific measurement. Epidemiologists should therefore seek to answer (a) the importance (if any) of inhaling, (b) whether the claimed increase of lung cancer from smoking is a steady cumulative process or whether there is a threshold up to which no increase is significant, and (c) the relative aetiological potency of smoking versus age, sex, air pollution, previous infection, genetic factors and so forth. Many other factors should not be overlooked, *e.g.*, socio-economic features of life, malnutrition and dietary deficiencies, stress and strain, the decline in mortality from respiratory infections (in particular tuberculosis) and the psycho-emotional characteristics of smokers and non-smokers.

Austin Bradford Hill

BORNEFF, J. & FISCHER, R. Cancerogene Substanzen in Wasser und Boden. V. Untersuchungen an Filter-Aktivkohle. [**Carcinogenic Substances in Water and Soil. V. Investigation on Filter-Active Carbon**] *Arch. f. Hyg. u. Bakt.* 1961, Feb., v. 145, No. 1, 1–11, 6 figs. [16 refs.]

The English summary appended to the paper is as follows:—

"A fluorescent substance was obtained through extraction with benzene of 50 kilograms of adsorptive carbon adapted for filtration of water for domestic use. Because of the small quantity available (about 100 μg) and the absence of typical adsorption bands, the substance could not be analyzed chemically. The same substance was obtained in about equal quantity by treating the carbon with a solution of detergents. In all probability it is non-cancerogenic. As the quantities involved are very small they present no health hazard even if water intended for filtration should contain detergents. Adsorptive carbon may therefore safely be used as a filter."

BORNEFF, J. & FISCHER, R. Cancerogene Substanzen in Wasser und Boden. VI. Zum Nachweis polycyclischer Kohlenwasserstoffe mittels spektraler Fluoreszenzzerlegung. [**Carcinogenic Substances in Water and Soil. VI. Spectrum Fluorescent Analysis of Polycyclic Hydrocarbons**] *Arch. f. Hyg. u. Bakt.* 1961, June, v. 145, No. 4, 241–55, 10 figs. [10 refs.]

This is the sixth in a series of papers on carcinogenic substances which may be present in water and in soil [this *Bulletin*, 1959, v. 34, 1123; 1960, v. 35, 9, 1077, and above]. It contains a very detailed technical account of the methods of analysis of some of these substances by means of the Zeiss-Spektralphotometer PMQ II. The qualitative and quantitative data obtained are fully described and discussed with graphs to illustrate the findings.

The substances examined by the method of measurement of spectral fluorescent decomposition were anthracene; 1,12-benzperylene; 1,2,-benzpyrene; 3, 4-benzpyrene; chrysene; 1, 2, 5, 6-dipenzanthracene; fluoranthene; perylene; and pyrene. *M. E. Delafield*

MISCELLANEOUS DISEASES

TAKAMURA, T. & UEDA, S. **Hematologic Findings in Children exposed to A-Bomb Radiation in Utero in Hiroshima.** *Blood.* 1961, June, v. 17, No. 6, 728–37, 7 figs. [19 refs.]

"1. Hematologic findings in in utero atomic bomb irradiated and nonirradiated children of Hiroshima between the years 1950 to 1957 have been compared and no changes peculiar to the irradiated group were found.

"2. Despite several predisposing factors in Japan toward the development of iron deficiency in children overt anemia was rare and distribution of hemoglobin levels was only slightly lower than those reported in American and European children.

"3. Leukocyte levels, irrespective of age of the children, were found to be progressively falling in the children of Hiroshima, whether or not exposure to irradiation had occurred. Thus, by 1957 the values were distinctly lower than those reported for normal children in Japan and the United States. No cause for this change was apparent."

Sanitation

The order in this section is:—**General; Water and Swimming Baths; Sewage, Trade Waste and Refuse Disposal; Housing; Infestation; Atmospheric Pollution; Port, Ship and Aviation Hygiene.**

WATER AND SWIMMING BATHS

(Including the Bacteriology of Water Supplies)

FIGURA, V. Una nuova tecnica di prelevamento di campioni di acqua in profondità in fiumi, laghi, mari per ricerche batteriologiche e chimiche. **[A New Technique for Obtaining Water Samples at any Depth from Rivers, Lakes and Sea for Analysis]** *Nuovi Ann. d'Igiene e Microbiol.* 1961, Jan.–Feb., v. 12, No. 1, 58–71, 2 figs.

The English summary appended to the paper is as follows:—

"A new method for drawing up water samples at any depth and in any quantity from lakes, rivers and seas for chemical and bacteriological tests is described.

"Such a method does not use a new instrument, but is based on the employment of an already known equipment: a small hand pump, a diver's hose as long as the desired depth, a non-return valve placed at the free end of the hose, and a lead ballast. At the time of the drawing up, pump hoses and ballast are extemporally disinfected with a solution of sodium hypochlorite at 1% of active chlorine. In order to avoid the filling of the diver's hose with water of the superficial and intermediate layers during immersion, the same hose is dipped, full of a disinfectant solution kept in by the non-return valve and by the counter-pressure from below to above.

"By successive pumping, the hypochlorite disinfectant solution is eliminated (control by the o-tolidine reaction), and, when one is sure that the upcoming water does not contain any more active chlorine and that it has the same temperature of the tested layer, samples of the upcoming water are taken; in such a manner a direct communication is created for the sampling of deep water which excludes water of superficial and intermediate layers.

"The advantages are several: 1) this method is valid for any depth and does not require special instruments which are often complicated and inefficient for the remote control of the containers' lids (messenger loads, propellers, electromagnets, etc.); 2) the water may be drawn in any quantities directly from the pump to the ground or on the boat; 3) the extemporary disinfection allows repeated drawing-ups at short intervals of time; 4) the equipment is very simple and solid and can be left in place at the same depth with special moorings, for drawing up water at intervals of time."

PINTUS, L. Ricerche comparative su alcuni metodi per la dimostrazione degli indici di inquinamento fecale nelle acque destinate ad uso potabile. **[Comparative Studies on some Methods for demonstrating the Indices of Faecal Contamination in Water Intended for Drinking Purposes]** *Igiene Moderna* 1960, Nov.–Dec., v. 53, Nos. 11/12, 664–86. [1 refs.] English summary (7 lines).

The author has been carrying out bacteriologic examination of samples from a surface water supp which is subjected to prechlorination, coagulation wi aluminium sulphate and settling, chlorination, filtratic and yet a final chlorination. He has been testing f coliform organisms and enterococci and for *Clostridiu welchii* as indicators of faecal pollution and he stat briefly the technique which he has followed. H tabulates, and comments on, his results from the examin tion of 100 samples of the raw water and 40 sampl (in the same series) taken immediately after the initi prechlorination and then again at the end of the f treatment.

He concludes that, in raw water, his tests for coliforms proved more sensitive than those for enterococci, and that the test for *Cl. welchii* was the least sensitive. In samples of partially and of fully treated water the search for *Cl. welchii* proved the most sensitive of the 3 tests, and this is attributed to spore resistance.

The ideal to be aimed at is the absence of *Escherichia coli* in 100 cc. of water, if this is intended for drinking purposes.

Generally speaking, the coliform organisms and enterococci suggest recent pollution, whereas the contamination indicated by the presence of *Cl. welchii* suggests a more remote origin. *J. Cauchi*

AMERICAN PUBLIC HEALTH ASSOCIATION. **Standard Methods for Examination of Water and Waste-Water including Bottom Sediments and Sludges.**

This book was reviewed on p. 823.

MCCANN, M. B. **Mishap at a Town's Reservoir—Remedial Works and Preventive Measures.** *Monthly Bull. Ministry of Health & Pub. Health Lab. Service (directed by Med. Res. Council).* 1961, July, v. 20, 122–4.

In March 1961 a water crisis arose in the Borough of Worksop because the main water reservoir had developed an extensive leak. This reservoir, built about 80 years ago when little was known about reinforced concrete, had a floor only 2½ inches thick. On inspection it was found that a section of the floor 6 ft. by 4 ft. in size had disappeared. Water was pouring through this hole into the fissured sandstone substratum. It became necessary to obtain the services of a cementation company to drill the entire floor at 6 ft. intervals and pump in quick-drying liquid cement at high pressure to fill the extensive cracks and fissures that were found.

There is a good account of the active measures that were taken in this emergency to ensure the safety of the water supply. This supply comes from 4 deep boreholes and normally is pumped into the reservoir. An additional supply of water comes from a colliery. Water during the emergency was pumped directly into the main and chlorinated at a dosage stepped up from the usual 0·4 to 2·0 parts per million. The difficulties which occurred over this improvised chlorination are described.

The energetic action of the public health authorities was effective for no water-borne infection occurred in the town after the failure of the reservoir. The only casualties reported were among goldfish in various parts of the town. *M. E. Delafield*

MARSHALL, J. D., WOLFORD, Claire B. & FABER, J. E. **Characterization of *A. faecalis* isolated from an Iodine-Disinfected Swimming Pool.** *Pub. Health Rep.* Wash. 1961, June, v. 76, No. 6, 529–33, 1 fig.

An organism comprising approximately 99% of the total bacterial count of a swimming pool water was identified as *Alcaligenes faecalis* [*Bact. alkaligenes*]. Neither coliform organisms nor faecal streptococci could be found in the water which was disinfected with iodine. No adverse effects were observed on swimmers when the pool water contained as many as 6,000 *Alcaligenes faecalis* organisms per millilitre.

One of the most important results of this study was the discovery that this organism has extremely high resistance to halogen disinfectants. For example, free chlorine in concentration of at least 0·75 part per million was required to kill 100% of the organisms within 10 minutes and with combined chlorine a dose of over 19 p.p.m. was required, and in the case of iodine over 2·5 p.p.m. was required.

It would appear that *Alcaligenes faecalis* is of no great significance from the public health point of view in swimming pool water, but if possible the source of infection should be traced and attempts made towards its eradication. *E. Windle Taylor*

SEWAGE, TRADE WASTE AND REFUSE DISPOSAL

MINISTRY OF HOUSING & LOCAL GOVERNMENT. **Fourth Progress Report of the Standing Technical Committee on Synthetic Detergents** [CREMER, H. W., Chairman]. 19 pp., 2 charts. 1961. London: H. M. Stationery Office. [1s. 6d.]

This interesting Progress Report contains much information on the field and laboratory work which has been done on the comparison of biologically "hard" and "soft" detergents. The soft detergent of present interest is Dobane JN.

The trial of the biologically soft detergent in the Luton area [this *Bulletin*, 1960, v. 35, 749, 1082] has continued and although as yet it has not been practicable to replace more than 80% of the hard by the soft detergent, the experimental distribution of the new material has been considerably extended to other areas. From the reports it is clear that "There has been a reduction in the concentration of synthetic detergents present in the effluents from sewage works and in the watercourses into which they discharge in the South of England" but elsewhere the general situation is unchanged. It is stated that "The removal of 80% of the soft detergent appears to be relatively easy and is achieved, or approached, even where the treatment is such that the removal of hard material is substantially less than 68%".

There is an account of the analytical investigations which have been carried out and have yielded important results. These are summarized:—"Recent work has demonstrated the presence in sewage effluents of

residual breakdown products from synthetic detergents which are not indicated or measured by the analytical methods at present in use". Until more is known about these products no firm conclusions on the ultimate value of the new soft detergent can be reached. The Committee is of opinion that "further improvement of the synthetic detergent now in use, or the development of a new active agent, is necessary". *M. E. Delafield*

TOKUOMI, H., OKAJIMA, T., KANAI, J., TSUNODA, M., ICHIYASU, Y., MISUMI, H., SHIMOMURA, K. & TAKABA, M. **Minamata Disease.** *World Neurology.* Minneapolis. 1961, June, v. 2, No. 6, 536–45, 6 figs. [17 refs.]

"Minamata disease is a mysterious neurologic illness which has occurred since 1953 in Minamata City located on Minamata Bay in southern Japan." Research on the disease has been conducted since 1956 by Kumamoto University workers. The present paper contains an account (based on a 4-year study) of the epidemiology, aetiology and clinical features of the disease.

The clinical characteristics were observed in detail in 34 patients out of a total of 83 who are known to have been affected. The commonest form of the disease (19 cases) exhibited numbness of limbs and lips, concentric constriction of visual fields, ataxia, tremor and slight mental disturbance. In this type slow improvemen followed with some "neurologic sequelae". An acut fulminant form occurred in 7 patients all of whom die usually within 2 months. Chronic forms of the disease with irritative phenomena (5 cases) or contractures (3 cases) were also observed. The symptoms, clinical signs and findings of laboratory studies are fully described. In general the prognosis is poor and in the total of 83 cases there were 33 deaths.

It was noted that all the 83 patients frequently ate fish and that 57 of them were fishermen or members of their families. A similar disease was found to occur in cats in the area and feeding experiments on cats confirmed that fish and shellfish caught in Minamata Bay were toxic. There is a large chemical factory from which effluent flows into the bay and various theories about the poison in the fish were suggested. Hence experiments were made to test the possibility that manganese, thallium, selenium or other metals might be the toxic agent. The only positive evidence obtained in the search for the aetiological agent has led to a mercury poisoning theory. This evidence consisted of the finding that the mercury content of the silt, fish and shellfish taken from the bay and in the brain, liver, kidney and urine of the patients was exceedingly high. The main clinical features of Minamata disease including the constriction of the visual fields are similar to those which have been reported in the literature to occur in cases of alkyl mercury poisoning. *M. E. Delafield*

ATMOSPHERIC POLLUTION

WORLD HEALTH ORGANIZATION: MONOGRAPH SERIES No. 46. 442 pp., illustrated. **Air Pollution.** 1961. Geneva: Palais des Nations. [Sales Agent for U.K., H.M. Stationery Office.] [40s.; $8.00; Sw.fr. 24.–.]

This book is apparently intended as a survey of air pollution, for those who have to deal with it seriously for the first time. Each aspect is treated by a different expert or group of experts and as a result the whole suffers from the unevenness of quality of the parts, but on the whole it serves its purpose well. It should be of great value to municipal and public health authorities in the many towns all over the world which are suddenly expanding rapidly and facing an air pollution problem for the first time, or which are discovering that it is both possible and within their economic capacity to banish an old one.

In an introductory historical review E. C. HALLIDAY (pp. 9–37) shows how interest in air pollution has developed over the past 70 years and in particular since 1945. He also draws attention to the differences of attitude towards it in different countries. The second chapter is on *The identification of the air pollution problem*, in which Louis C. MCCABE (pp. 39–47) outlines very briefly the problems involved. Harry WEXLER's chapter on *The role of meteorology in air pollution* (pp. 49–61) is also very short, but provides an extraordinarily thorough and admirably lucid summary.

F. CAMBI's contribution entitled *Sampling, analysis and instrumentation in the field of air pollution* (pp. 63–96) is the most disappointing, since it includes only the very briefest mention, without references, of large numbers of methods, many of them standard techniques only incidentally used in air pollution studies; and surely not even the most enthusiastic empire builder among air pollution experts would claim "proton beams, electron beams, slow neutrons and fast neutrons" for his domain?

Morris KATZ's long paper on *Some aspects of the physical and chemical nature of air pollution* (pp. 97–158) is an extremely informative summary of facts and figures. It begins with quantitative data on emission in United States and United Kingdom towns, and then gives extensive figures on individual pollutants and sources in Los Angeles County and in the Greater Detroit–Windsor area. Particulates and gases are considered separately, and in particular the physical properties and chemical composition of the former are very well treated.

The chapter on *Effects of air pollution on human health* (pp. 159–220), by Harry HEIMANN, begins with a fairly long description of the major episodes in the past 40 years, including an extremely interesting table (p. 180) in which the main features of the three major disasters in the Meuse Valley, at Donora and at London are

compared. The author then gives a very sensible assessment of the few facts and extensive speculations which constitute our knowledge of the medical effects of air pollution.

E. J. CATCOTT's contribution on *Effects of air pollution on animals* is very short (pp. 221–231), but is followed by a long discussion on *Effects of air pollution on plants*, by Moyer D. THOMAS (pp. 233–278). Outside the United States this subject has received far less attention than it merits, so that the work described is almost entirely American and very largely concerns the effects found near Los Angeles. Despite these unavoidable geographical limitations the author has provided an extremely interesting report, beautifully illustrated with colour photographs, and very well worth reading. It may however be too detailed for many readers of this *Bulletin*.

E. LECLERC's very brief discussion of *Economic and social aspects of air pollution* (pp. 279–291) is followed by another short paper on the *Control of air pollution by site selection and zoning* by J. R. TAYLOR, A. HASEGAWA and L. A. CHAMBERS (pp. 293–306). Both of these are interesting but it is unfortunate that the authors of the latter largely confine themselves to the problems of Los Angeles, and only mention in passing the notable Russian work on these lines.

The prevention and control of air pollution by process changes or equipment is described by A. H. ROSE, D. G. STEPHAN and R. L. STENBURG (pp. 307–343). They outline clearly and concisely all the main methods, but their treatment of dust-collecting devices is open to the criticism that it does not sufficiently stress the relationship between particle size and the efficiency of the cleaning device.

K. BARKER and W. A. MACFARLANE's chapter on *Fuel selection and utilization* (pp. 345–363) mainly concerns the latter, and the treatment is almost entirely in terms of British experience. *Air pollution legislation: standards and enforcement* (pp. 365–380) is discussed by A. PARKER, who is particularly interested in legislation in Great Britain (which he is mistaken in identifying with the United Kingdom in this connexion, since there is no Clean Air Act in Northern Ireland), the United States of America and the Soviet Union. The contrasts in outlook are very interesting although the author does not discuss them: the British rely on nation-wide legislation, but it is up to the local authorities to enforce it and there is room for considerable latitude in enforcement: in the United States control is exercised under state or local powers and is often quite detailed: and in the Soviet Union it is based on a national "organizational structure". Legislation in other countries is also very briefly outlined.

There is a long final paper on *Radioactive pollution of the atmosphere* by H. P. JAMMET (pp. 381–415).

If this book is open to a general criticism it is on the grounds that the authors are not agreed among themselves as to what is meant by atmospheric pollution. This is unfortunate, for to the man in the street its meaning is fairly clear—it is the general contamination of the air he breathes in the street or in his home. It is a pity that some contributors should have taken the term to include types of contamination to which only those who work in particular industries are exposed normally. Nevertheless it is a really valuable review of the subject, well produced and indexed, and with the exception of Cambi all the contributors have provided adequate bibliographies. *John McK. Ellison*

AMDUR, Mary O. **The Response of Guinea Pigs to Inhalation of Formaldehyde and Formic Acid alone and with a Sodium Chloride Aerosol.** *Internat. J. Air Pollution.* London. 1960, Oct., v. 3, No. 4, 201–20, 13 figs. [13 refs.]

The author has described previously the technique she has developed using guineapigs to study the physiological effects of inhaling low concentrations of toxic gases and aerosols [this *Bulletin*, 1957, v. 32, 973]. The present paper continues this work; it is intended to be read in conjunction with another paper by GOETZ (to be published in *Internat. J. Air Pollution*), the two dealing with the toxicological trials and the physical theory respectively.

The measurements needed are intrapleural pressure, tidal volume, and rate of flow in and out of the lungs: from the first and third of these pulmonary flow resistance can be calculated, and this is the figure mainly used to express results. With 1-hour exposures to formaldehyde at concentrations less than 11 p.p.m. recovery was complete in 1 hour, but at 49 p.p.m. it was not, although the resistance to gas flow had dropped markedly from the high values reached during exposure. After 4 hours at 50 p.p.m. recovery was very slow, and was incomplete 2 hours later. Formaldehyde caused a small but significant increase in resistive work of breathing, estimated by the formulae given by OTIS *et al.*, (*J. Applied Physiol.*, 1950, v. 2, 592), as the author has consistently found with all the irritants she has studied previously.

For a very soluble gas like formaldehyde a considerable part of what is inhaled is removed in the upper respiratory tract. The protective effect which this affords for the lung was studied by inserting a polythene cannula in the trachea, so by-passing the scrubbing effect. This increased the effect of a given concentration of formaldehyde but did not alter the main features of the changes.

Formic acid produced broadly similar effects, but is more irritant.

Sodium chloride aerosols alone produced no substantial changes, but when given with formaldehyde the increase in resistance and decrease of compliance, and also the increase of resistive work, were consistently and significantly greater than those produced by formaldehyde alone, and the effects were produced at much lower concentrations: here again the effect was greater in animals breathing through a cannula in the trachea. The results are explained in terms of VORWALD's hypothesis (*Proc. U.S. Technical Conference on Air Pollution*, 1952, New York, McGraw Hill, p. 485) that gas is adsorbed on the aerosol surface and thereby sets up high local concentrations of irritant, which produce a greater response than the same amount of free gas.

With formic acid the addition of NaCl aerosol did not produce a consistent increase in resistance, although recovery was delayed. *John McK. Ellison*

Occupational Hygiene

The order in this section is:—**General; Occupational Physiology; Occupational Diseases (Dust Diseases, Poisoning, Skin Diseases, Others); Occupational Psychology; Heating, Lighting and Ventilation.**

GENERAL

CHALLEN, P. J. R. **Slough Industrial Health Service. Report.** *Ann. Occup. Hyg.* London. 1961, June, v. 3, No. 4, 283–90, 2 figs.

The Occupational Hygiene Unit report is reproduced from the Annual Report of the Slough Industrial Health Service, 1959–60. The unit, one of the few in Britain, is prepared to investigate problems in any part of the country. Requests came from managements and trades unions as well as from professional sources. Slough Estates Limited gave much support, and income from outside is increasing, though too slowly. Facilities are being considered by a London Teaching Hospital and collaboration with the Noise Control Section of the National Engineering Laboratory, Glasgow, is hoped for if the cost can be met. Staff difficulties interrupted the unit's research on noise and even with the encouragement and grant given by the Medical Research Council it took time to recruit a technical assistant. The work done by industrial hygiene units with considerable staff in other countries is contrasted with the small demand in Britain, and the lack of trained staff and modern equipment. Unless demand increases the organization cannot continue without subsidy.

Tables list the investigations of complex problems from dusts, chemicals, lighting, heat and noise, sometimes presented at design stage. Sampling and analytical methods for oil-mists were developed with help from the London Hospital Department for Research in Industrial Medicine. In butt welding, though silencers had been fitted to air discharge nozzles, octave band analysis shows that ear muffs were necessary and better than ear plugs. Plasma jet welding also presents a hearing hazard. Punch press and automatic machine shops have dangerous noise levels and research on machine design is needed as well as on methods for suppressing noise at source.

A family doctor diagnosed trichloroethylene poisoning in a degreasing-tank operator who, after 13 years' work, began to have symptoms when the tank was moved and work increased. Air concentrations were found to have risen much above the high MAC of 200 p.p.m., ventilation at the new site was poor, and the substitute operator was found to have a confirmatory level of trichloroacetic acid in the urine. Periodic urinary estimations of this and trichloroethanol are being planned for such operators in member firms. *M. Patricia Fitzsimons*

SCHNEIDER, R. F. **A Review of the Value of Periodic Health Examinations.** *Trans. Ass. Indust. Med. Officers.* 1961, Apr., v. 11, No. 1, 27–36, 2 figs. [41 refs.]

There has been much discussion in recent years concerning the value or not of periodic medical examinations as a health maintenance procedure. Schneider, whose approach is that of the personal physician, has presented a very strong case in their favour in this publication.

The idea is not new, and a system of periodic examinations for all persons and their children was recommended by a Dr. Horace DOBELL in England in 1861. The fact, that in 1961 there is still wide disagreement concerning the value of such examinations, suggests that the data accumulated so far are not adequate for an evaluation, and that there is a need for accurate criteria for assessing the data which are accumulated during such procedures.

In his discussion the author offers supporting evidence from his own experience as well as from that of other physicians, and while admitting the many limitations of periodic examinations, suggests that their performance does offer the following favourable results:

1. Previously unrecognized disease is detected, often while still asymptomatic and more likely to respond favourably to treatment.

2. Disease previously known to exist is found not to be receiving needed observation or treatment, and the patient can often be persuaded to seek and accept that which is required.

3. Certain base-line observations can be made and adequately recorded, facilitating the later recognition of changes when the appreciation of these may aid in the solution of diagnostic problems.

4. The contacts between patients and examining physicians offer a medium for highly personalized health education.

5. Reassurance concerning their health status is often a source of major relief and support to individuals with open or concealed anxiety about it.

6. Analysis of the results of many such examinations over a period of years would enable the making of many observations concerning factors of apparent importance in the maintenance of health and in the cause, prevention, early diagnosis, progress and treatment of various diseases. *P. J. R. Challen*

JONES, W. T. **Doubts on the Periodic Health Examination.** *Trans. Ass. Indust. Med. Officers.* 1961, Apr., v. 11, No. 1, 37–8. [21 refs.]

[Schneider (see above) is an obvious enthusiast, but not a blind one. The scepticism of the value of periodic medical examinations expressed by Jones in this paper ought to be present in the minds of all physicians conducting such examinations; otherwise it is inevitable that the examinations will develop into a sterile routine, and both the examiner and the examined will be lulled into a state of erroneous belief and sham security.] In his role as sceptic the author has rightly adopted the epidemiological approach and has stressed the need for critical evaluation of methods and results. More knowledge is

needed about the early clinical forms of disease and particularly about the earliest biochemical changes which may be detectable by routine "screening" procedures.

He suggests that it is upon population groups which are shown by epidemiological and other methods to have unfavourable health experiences that routine periodical medical examinations should primarily be concentrated. He concludes with the following statements: "Whilst we await basic advances in knowledge it is felt that our efforts in the field should be directed towards the better education of our patients. An awareness and willingness to recognize and admit early symptomatic deviations from health and a readiness to seek prompt advice, against a background of a mature mental state, would seem to be the most rational and rewarding approach for at least the immediate future". [This is sound thinking and policy but it does not exclude the dual approach, and provided that periodical medical examinations are carried out with ample time, care and thought, there is no fundamental reason why they should not provide information which could be intrinsically valuable to preventive medicine.] *P. J. R. Challen*

FORDYCE, I. D. **Olfaction Tests.** *Brit. J. Indust. Med.* 1961, July, v. 18, No. 3, 213–15.

A test of the sense of smell was repeated in 100 persons to see whether the method gave consistent results. PROETZ's (*Essays on the Applied Physiology of the Nose*, 1941, St. Louis, U.S.A., Annals Publishing Co.) solution of phenol in liquid paraffin, sp. gr. 0·880, was used in 8 concentrations. Grade 1 contained 0·0192 gm. phenol per litre, and the others 2, 5, 10, 25, 50, 100 or 200 times this amount. Each test was begun with the weakest solution and continued in order until smell was noticed. Results were discarded in 2 subjects; 1 gave oscillating answers, positive at 2 and negative at 200 olfacts, the other developed a cold. Of the 98 subjects, 79 repeated their initial result to within plus or minus 1 grade, independently of the observer, initial grade, age, smoking habits or interval—a few hours to 12 days—or, in 35 subjects, at a third test after 3 weeks. Grading improved sometimes with experience.

The acuity of smell was also tested in 147 subjects in relation to smoking habits and age: 90 did not smoke, 46 smoked cigarettes, 10 a pipe and 1 cigars. There was no difference between cigarette smokers and non-smokers. The 11 other smokers showed a possibly decreased acuity. They, and also 1 woman with anosmia, were excluded from the sample to test the effect of age. Acuity decreased with age in these 135 persons, but the small 35–44 year-group was more affected than older groups.

M. Patricia Fitzsimons

ADAMS, R. G. & CRABTREE, N. **Anosmia in Alkaline Battery Workers.** *Brit. J. Indust. Med.* 1961, July, v. 18, No. 3, 216–21.

Nasal examinations are described and the results show that the pathological changes found are of no value in assessing the degree of loss of the sense of smell.

The olfactory acuity was assessed by the phenol-smelling test [see above] and also by questioning 106 workmen engaged in battery manufacture who were exposed to cadmium and nickel dust, and 84 controls, matched for age. The cadmium powder contained 60–64% cadmium oxide and 16–18% ferrous iron, the nickel powder, 72% nickel hydroxide and 18–20% graphite. Atmospheric concentrations show cadmium values to be variable and higher than nickel. A phenol-test grade of more than 200 olfacts was recorded as anosmia and an answer signifying that the sense of smell was lost was accepted as reported anosmia.

Anosmia was reported by 15% of the 106 workers and recorded in 27·3%, including the 15%; control figures were zero and 4·8%. Diminished smell-sense was reported by 23 workers; in 11 of them anosmia was recorded; control figures were 17 and 3. As evidence of the difficulty of quantitatively assessing hyposmia, widely differing grades were recorded in the remaining 12 of the 23.

Proteinuria was found in 17 workers. Anosmia was recorded in 11, and 12 reported anosmia or hyposmia; figures for the 89 without proteinuria were 18 and 27. Among 28 older workers, exposed to more dusty conditions in a similar factory closed 13 years before, 13 had a grade of 200 compared with 16 of the remaining 78, and 13 of the 17 with proteinuria had worked there. Age is unlikely to account for the great difference. Exposure to the dusts ranged from a few weeks to more than 30 years.

The nose was examined in 85 workers and 75 controls. In 1 case only, the atropy and crusting extended to the olfactory area in the upper third. Biopsy in a known battery worker showed a mild, non-specific, chronic inflammation of the submucosa with focal lymphocytes and scattered eosinophils. No changes ascribable to an irritant were found in 36 workers and 45 controls. Signs of non-specific irritation were more frequent in the workers, but no significant relationship was found between this appearance and recorded or reported anosmia.

Hyposmia has not been reported from iron oxide or graphite or, except recently in Russia during nickel production by an electrolytic process, from nickel. It is concluded that the anosmia was due to exposure to cadmium or nickel dust or to a mixture. The positive correlation with the proteinuria suggests that cadmium oxide is the offending dust, but it may well be that any irritant breathed for long periods as dust or aerosol may impair the sense of smell. *M. Patricia Fitzsimons*

CORNWALL, C. J. & RAFFLE, P. A. B. **Sickness Absence of Women Bus Conductors in London Transport (1953–1957).** *Brit. J. Indust. Med.* 1961, July, v. 18, No. 3, 197–212, 19 graphs.

For absences lasting 4 or more days, all of which are supported by a medical certificate, the experience of single and married women conductors employed by London Transport is compared. The comparisons are given in detail in 22 tables and 19 sets of diagrams [which need to be consulted] in which are shown for all causes of absence, for 20 broad diagnostic groups and in 5-year age-groups: (a) the exposure to risk, (b) the number of days of sickness absence, (c) the average annual duration of absence per person, (d) the number of spells with (e) their annual inception rate, and (f) their average

length. For all causes of sickness taken together, married women show an excess of about 40% in number of spells and about 60% in days of absence. For most of the separate diagnostic groups the differences are of this same high order. The excess in married women would therefore seem to be of social origin.

Austin Bradford Hill

JUNG, H. D. Ursachen und ökonomische Bedeutung der Unterleibserkrankungen bei landwirtschaftlichen Arbeiterinnen im Heim für soziale Betreuung. **[Causes and Economic Significance of Abdominal Disorders in Women engaged in Agricultural Work at a Home for Social Welfare]** *Ztschr. f.d.g. Hyg. u. ihre Grenzgebiete.* Berlin. 1961, June, v. 7, No. 6, 448–55, 5 figs.

This paper from East Germany contains an account of the abdominal disorders and resulting absence from work experienced by certain women who were condemned to legal punishment and for this reason were sent to a rehabilitation home. This home, the purpose of which was by social education to render the women fit to return to the community, could accommodate 52 women, who were sent out to work at a neighbouring farm.

The investigation, which was particularly undertaken because of the high sickness absence from work of these women, and is described in detail, showed that the main primary cause was venereal disease. About 50% of the women had had gonorrhoea before admission to the home and a proportion of these, while doing land work, suffered from secondary effects such as salpingitis and metritis. Another cause of abdominal disorders in these women is firmly emphasied. This is that the women coming straight from prison to the home were not provided with adequate protective clothing and shoes by the farm management and the home, who should have obtained these according to a legal schedule.

It is stated that the economic loss in gross production at the nationalized farm in 1959 caused by inflammatory abdominal diseases of the female outdoor workers amounted to 14,000 DM. *M. E. Delafield*

POSSNER, W. Ergebnisse arbeitshygienischer Untersuchungen bei Ultraschall-Materialprüfern. **[Industrial Hygiene in Relation to Workers in the Supersonic Testing of Materials]** *Ztschr. f.d.g. Hyg. u. ihre Grenzgebiete.* Berlin. 1961, June, v. 7, No. 6, 420–36, 11 figs. [11 refs.]

This account of an enquiry into the possible hazards to the health of 125 workers engaged in the testing of materials by supersonic waves is preceded by a technical description, with diagrams and photographs, of the particular supersonic test apparatus used by these workers, which is of a piezo-electric type. There is a description of the way in which this test apparatus is used. Reference is also made to published reports on the signs observed and symptoms complained of by workers testing with supersonic waves.

Details of the age and duration of work of these 125 workers, of whom 10 were women, are given.

The results of medical investigation are described at very great length with tables and with diagrams to illustrate the frequency of symptoms in relation to hours of work or duration of employment.

In summary, the findings were:—(1) Skin disease on hands and fingers—vesicular-papular eczema was observed in 7 workers, scaliness of skin in 4, and 2 complained of interdigital itching; these skin troubles are stated to be the result of exposure to oil and dirt. (2) Complaints of various kinds made by 29 men and 6 women included pain, weakness, feelings of cramp and paraesthesia in the hands and arms. A few workers complained of eye-strain. *M. E. Delafield*

GOVONI, L. Lesioni caratteristiche nelle mani dei mugnai addetti alla rabbigliatura delle macine gli "acciarini". **[Steel Spicules: Characteristic Lesions in the Hands of Millers engaged on the Reconditioning of Mill Stones]** *Acta Med. Legalis et Socialis.* Liège. 1959, July–Dec., v. 12, Nos. 3/4, 107–17, 11 figs. English summary.

The case is described of a man aged 44 years who came under treatment as a result of an injury sustained at work involving the right index finger. An X-ray of the hand was taken and a most curious condition quite unrelated to the accident came to light. All the soft tissues of the little, ring, middle and to a lesser extent of the index finger, were absolutely packed and studded with innumerable, minute, metallic, foreign bodies lying at various depths. The greater number of these tiny splinters of various shapes were found in relation to the proximal half of the fingers on their dorsal aspect. X-ray of the left hand revealed a still greater number of similar foreign bodies to a total of some hundreds. The hands were otherwise featureless except for the fact that a brownish punctate appearance of the skin was present over the dorsal surface of the 3rd and 4th metacorpo-phalangeal joints of the left hand, and there was a slight diminution in elasticity in the affected areas.

This man had worked since he was 12 years old on the task of reconditioning mill stones which are made from sandstone containing 60% to 90% silica. There is accordingly a silicosis risk which is well recognized and is mentioned merely in passing. The job consists of making channels and tracks on the grinding faces of new stones and making good those which have become worn with use. The work is carried out entirely by hand, and the tools which give rise to these minute splinters are a hammer and a chipping hammer, and a sort of cold chisel, and when the surface of the stone is struck small fragments of metal of many different shapes fly off at considerable speed. These are what the workmen call *acciarini* (steel spicules). The greatest concentration of spicules occurs at certain sites as a result of the manner in which the tools are held in the hand. All the men who undertake this kind of work know about the lesions due to these steel spicules, and although at first they are painful and sometimes cause loss of time, the men say that their hands soon get accustomed to the multiple, small, foreign bodies. Protective gloves only make the lesions worse because the splinters pass through the leather with ease and embed themselves in the same situation, and infection frequently follows.

Although yellow macules and tattooing of the hands have previously been described in millers, and the tattooing has been considered occupational in origin, the brownish discoloration described in this paper seems quite different from either of these conditions. The author thinks that the occurrence of these metal splinters in the hands of these men must be accepted as an occupational stigma although they themselves usually regard them as a normal accompaniment of their work. Obligatory antitetanic prophylaxis is advocated.

[It sounds as though the steel used in the hammers and chipping hammers may be too hard, which would make them fragment rather easily. If the workers temper their own tools, which is very likely in a rural industry, they may have rendered them over-brittle.]

G. F. Keatinge

OCCUPATIONAL PHYSIOLOGY

GAMBASHIDZE, G. M. **[The Significance of Diurnal Periodicity of Physiological Functions in evaluating Efficiency of Workers in Industries with Three-Shift Operation]** *Gigiena Truda i Professional'nye Zabolevaniya.* Moscow. 1961, v. 5, No. 6, 13–18, 3 figs. [17 refs.] [In Russian.] English summary.

The investigation was carried out on 15 operatives at a mechanical bakery working a 3-shift (8-hour) cycle, rotating every 6 days. They had no meal break during a shift. The work was heavy, moving about 10–12 tons during a shift and was carried out at an air temperature of 24°–29°C. and a humidity of 72–81%. The work was the same on all shifts.

Measurements were made every 2 hours of body temperature, pulse rate, blood pressure, muscle strength, muscle endurance and the latent period of motor reaction, in order to observe the effect on such functions of both work and diurnal rhythm. A table of the average values on each of the 3 shifts shows that most indices were lower on the night than on the morning or afternoon shift. There was a steady fall of muscle endurance on all 3 shifts, attributed to the strenuous character of the work. But a diurnal effect was also distinguished, at midnight (beginning of the night shift) the initial level was lower than on the other shifts and the endurance on the night shift fell until 4 a.m., after which it remained almost constant. The body temperature followed its usual diurnal course (despite the hot conditions and the physical effort of the work).

Comparison of results at the beginning and end of a week shows that the curve for muscle endurance is set at a lower level on the 5th night than on the 1st, whereas such an effect was not apparent on the morning and afternoon shift. It is claimed that this is a physiological demonstration that industrial fatigue shows up more readily on the night shift.

Studies were made on 7 workers at work and at home, including both a day shift and a night shift. The diurnal temperature curve is lower when the worker is on the night shift [the whole curve is about 0·2°C. lower, the shapes of the curves are virtually identical].

W. R. Lee

OCCUPATIONAL DISEASES

FAY, J. W. J. & ASHFORD, J. R. **A Survey of the Methods developed in the National Coal Board's Pneumoconiosis Field Research for correlating Environmental Exposure with Medical Condition.** *Brit. J. Indust. Med.* 1961, July, v. 18, No. 3, 175–96, 7 figs. [29 refs.]

The Pneumoconiosis Field Research Unit of the National Coal Board is developing methods of estimating direct relationships between the radiological abnormality associated with simple pneumoconiosis and single measures of past environmental hazard. Some of these methods are described in terms of biological assays estimating dose-response (*i.e.*, environmental hazard—radiological abnormality) relationships. The population from which the Unit draws data consists of about 35,000 surface and underground workers at 25 collieries where radiological surveys have been carried out about once every 4 years since 1953. A continuous programme of dust measurement at each colliery since 1953 permits matching of the radiological change and dust exposure of each man since 1953; in addition the earlier occupational history of the workers is available. The medical and environmental data are described in detail and some observations are made about sources of error and bias and the difficulties which arise in their use.

The classification of workers into a small number of groups defined by the few recognized categories of pneumoconiosis is a major difficulty in obtaining a useful relationship and the first development was the derivation of an underlying continuous scale of radiological abnormality. Thus, by using the internationally agreed categories 0 (" within normal limits "), 1, 2 and 3 for pneumoconiosis, 2 continuous variates were derived to estimate response as the probability that an X-ray film will be read as (1) category 1 or more and (2) category 2 or more. Transformation of these probabilities to probits plotted against the logarithm of period of exposure (in this case the period spent in the occupation) for 371 men gave 2 parallel " dose-response " lines. From such analysis the

usual advantages of relationship between 2 quantitative variates, over that between one quantitative and one qualitative, emerge both for estimates on a single sample and for comparisons between samples of workers.

The 371 men used in this analysis were all drawn from one colliery and all had only one specified occupation, so that the "dosage" could be reasonably estimated. The second development was concerned with the more usual situation in which many of the workers had a variety of jobs during their past occupational history. At one colliery data relating to some 1,700 men were considered; 2 dose-response lines were calculated as before for each of 3 periods of exposure defined by type of work (coal getting, preparation and elsewhere underground). For each occupation the response lines for categories 1 or more and 2 or more were again parallel, although the fact that the pair of lines for the preparation shift was much steeper and crossed the other 2 pairs complicates interpretation; it is suggested that period of exposure and dust hazard were not similarly related in all jobs.

The third development described makes use of the matched data available since 1953. Various ways of using these data are mentioned and in a practical application results of 485 men at the first two surveys in one colliery were used. The 2 sets of films were read independently (although the Unit is carrying out trials of "independent" versus "side by side" readings) and a check sample of the earlier set was read at the time of the second survey; this established no material change in the levels of classification. For each man the data available were the 2 independent X-ray classifications in the usual 4 categories of pneumoconiosis, a summary of his occupational history and an unbiased estimate of his dust exposure between the 2 surveys. The men were classified into 9 sequences of pairs of film readings so that "no change" included (0, 0), (1, 1), and (2 or more, 2 or more), "apparent progression" included (0, 1), (0, 2 or more) and (1, 2 or more) and "apparent regression" included (1, 0), (2 or more, 0) and (2 or more, 1). The probability that any of these pairs of readings would be obtained was then expressed as a function of the 2 environmental variables by the methods already described limiting past occupational history to the period spent in "dusty" occupations before the first X-ray survey. The possible interpretations of the results of this analysis are discussed, but because of the high initial prevalence of pneumoconiosis, there was relatively little change in classification within the limits set (57 had apparent progression and 43 apparent regression) so that the dust hazard actually measured between surveys accounted for only a small part of the total hazard to which the men were exposed. In this respect the method will probably be of more use after a longer period of observation in younger populations or in other fields of application.

E. A. Cheeseman

NEW ZEALAND DEPT. OF HEALTH. Med. Stats. Branch. **The Grey Valley Survey. Smoking, Lung Function and the Effects of Dust in Coal Miners in New Zealand** [DE HAMEL, F.A.]. 55 pp., 11 figs. [21 refs.] Special Rep. Ser. No. 3. 1961, Feb. Wellington: R.E. Owen, Govt. Printer. [4s.]

The Grey Valley on the West Coast of the South Island of New Zealand is a compact coal-mining area and for this reason it was chosen for a large-film radiographic survey in 1958. At that time 57 or more mines were being worked but only 5 of them employed more than 100 men: nevertheless with the support of the Miners' Unions an overall response rate of 85·5% was obtained: 1,231 men were employed underground (response rate 93·2%) and 339 were surface workers (response rate 57·8%). The smaller response on the part of the surface workers is attributed to the fact that their duties are largely clerical or peripatetic. It was impracticable to arrange a statistically ideal non-mining control group but men from the larger local employers were asked to volunteer and altogether 228 men made themselves available. The X-ray plant was housed in a large mobile van which was big enough to enable it to be used for interviews and lung function tests which were carried out in addition to $14'' \times 17''$ X-ray films of chest. A record was made of the working histories, smoking habits, symptoms and previous illnesses of those participating in the survey and for this purpose a suitably modified version of the College of General Practitioners' "Questionary on Respiratory Symptoms" was adopted for interviewing and recording answers. As a test of lung function the Forced Vital Capacity was estimated by means of a modified Gaensler type spirometer with a Venner $\frac{3}{4}$-second electronic time switch to terminate the FEV reading.

The greater part of the report consists of a series of admirable tables and figures, and in fact the text may be regarded simply as a running commentary on them, although without access to the store of information they provide some of its force is lost. Still, many interesting points emerge but in a short abstract it is only possible to deal with a few of them. In carrying out lung function tests it was found that a venturi effect could occur when the subject failed to close his lips securely round the mouthpiece, and this happens more frequently in older men who have removed their dentures and whose lips and mouth are thus rendered more flaccid. However, the amount of increase is not exactly repeatable and the error is noticed on subsequent tests. The usual relationship was found between smoking, particularly cigarette smoking, and bronchitis: however, there was strong evidence that dust from working underground in a coal-mine aggravates the symptoms of cough produced by cigarette smoking if the amount smoked is more than 20 cigarettes a day or more than 4 oz. a week. The highest proportion of normal X-ray findings occurs in the non-smoking group but the second highest is in the heavy cigarette smoking group. This finding is explicable on the grounds that only those who are respiratorily fit are likely to be heavy smokers which the author rightly thinks is a concept worth investigating.

The X-ray survey revealed a total of 32 cases of pneumoconiosis of category I or more (presumably on the basis of the International Classification suggested by the World Health Organization in Geneva in 1958 [this *Bulletin*, 1960, v. 35, 21]) giving an incidence of 2·1% in all miners and ex-miners examined: however, only 22 cases were found in present miners (1·6%). More than one-half the cases found were in category I. 6 men

were classified as simple category II, 3 as simple category III, 3 as IIA, 1 as IIIA, and 1 as IIIB. In all but 4 of the cases, the miners gave a history of having worked underground in overseas coal or other mines for an average period of 13 years each. Of the 4 who had not worked overseas 2 had spent 12 and 18 years respectively in New Zealand quartz mines, 1 had spent 6 years tunnelling as well as 14 years in coal, but the last had spent all his 46 years working life underground solely in New Zealand coal-mines. This last was simple category I and appears to be a conclusively indigenous case. However, of all those who had worked more than 30 years in New Zealand coal-mines underground 6·9% had evidence of pneumoconiosis. It seems therefore that pneumoconiosis can and does occur in the Grey Valley but is not likely to arise until after at least 30 years underground, and will probably be mild in degree. There was no case of PMF occurring in a miner who had worked solely in coal mines in New Zealand although it has occurred when a miner has also worked in quartz. The incidence of tuberculosis, either healed or otherwise, was not high enough on which to base valid conclusions: it was, however, lower in underground miners than in any other group.

[This survey is something of a *tour de force* since the interpretation of the X-ray films, the testing of lung function and the interviewing and recording of answers appear to have been done solely by the author. Apart from being a means of avoiding observer error it is a remarkable solo performance especially since the whole task was completed in 3 months.] *G. F. Keatinge*

THEODOS, P. A., CATHCART, R. T. & FRAIMOW, W. **Ischemic Necrosis in Anthracosilicosis.** *Arch. Environment. Health.* Chicago. 1961, June, v. 2, No. 6, 609–19, 7 figs. [11 refs.]

This paper opens with a brief review of recent literature dealing with the association of tuberculosis and conglomerate fibrotic masses in "anthracosilicosis" and it is stated that the presence of cavitation is usually considered sufficient evidence for the diagnosis of active tuberculosis even if a positive sputum cannot be obtained. On the other hand the view is expressed that when tuberculosis complicates "anthracosilicosis" the initial direct smear is positive for tubercle bacilli in 85% of cases [this *Bulletin*, 1952, v. 27, 569]. If a positive sputum cannot be obtained then other causes have to be considered and this study was undertaken to clarify the nature of cavitation and its relationship to tuberculosis.

The investigation was based on the observation of 1,980 anthracite coal-miners over 13 years. The patients were admitted to hospital for evaluation of their silicosis and for studies on the nature and degree of cardio-respiratory impairment. At the same time a detailed history was taken, followed by physical examination. Three X-rays of chest were taken (posterior-anterior in deep inspiration and deep expiration and lateral view) with tomograms if cavitation was doubtful and in some cases bronchograms were done. Bronchoscopy was performed in those cases with cavitation in which the sputum was negative or neoplasm suspected. Bronchial aspirates were examined routinely for acid-fast bacilli, neoplastic cells, fungi and other bacterial organisms. A routine complete blood count, urinalysis, fasting blood sugar, blood urea and nitrogen estimations, blood serology and erythrocyte sedimentation rate were carried out. The sputum expectorated in 24 hours was measured in gm.: if the initial examination revealed no acid-fast bacilli then 2 more direct smears with culture of one specimen, one 24-hour concentrate, one 72-hour concentrate, 3 examinations of the fasting gastric contents with culture of one specimen, and a smear and culture of the bronchial secretions when obtained were done. In the last 5 years only one direct smear, culture of two 24-hour concentrates and a direct smear and culture of the bronchial aspirate obtained on bronchoscopy were undertaken. Tuberculin and histoplasmin skin tests, the carbon dioxide combining power and an electro-cardiogram were also performed as a routine. In post-mortem examinations special attention was given to the area of cavitation and direct smear and culture of the contents and histological study of the cavity wall and surrounding tissue was carried out.

Cavities were seen in the X-rays of 206 cases (10·4%) and of these acid-fast bacilli were recovered in the sputum either by direct smear or culture in 141 (68·4%). Of the remaining 65 cases carcinoma of the lung accounted for cavitation in 2 cases and cystic bronchiectasis was present in 2 others. The cause of cavitation could not be determined in 4. In 57 cases (27·7%) in which the cause of cavitation is held by the authors to be best explained by ischaemic necrosis, X-ray evidence showed the cavitation to be unilateral in 37 and bilateral in 19 instances, and bilateral cavitation was discovered in one case only on post-mortem examination. The morbid anatomy of ischaemic necrosis is described and a series of microphotographs are used to illustrate the typical vascular changes with absence of giant cells and epithelial cells. The clinical course in patients with ischaemic necrosis is benign, rarely with evidence of fever, toxicity or generalized disability but frequently there is spontaneous expectoration of large amounts of inky-black material. Some of the literature dealing with the vexed question of the association of tuberculosis with conglomerate fibrosis, especially where cavitation is present, is reviewed and the conclusion is reached that in such cases "when cavitation occurs it is immediately attributed to tuberculosis". The authors, however, consider that cavitation can occur in "anthracosilicosis" in the absence of active pulmonary tuberculosis but by its benign course can be distinguished from that occurring as a result of tuberculous infection and they think that it is more common than is clinically appreciated. Recognition of this form of cavitation is important because of its prognostic therapeutic implications.

[85% of positive direct smears for tubercle bacilli in "anthracosilicosis" complicated by tuberculosis would be regarded by most British workers as a very high figure. Much space is given in this paper to attacking the view that massive areas of conglomerate fibrosis represent silicosis modified by tuberculosis yet the argument culminates in the statement that "In many patients the tuberculosis represents old burned-out disease that plays no clinical role, if indeed it were ever present". This is surely tilting at windmills. The views expressed on the morbid anatomy seem to have been based on a modicum of histological material.] *G. F. Keatinge*

NAKAMURA, T., TAKIZAWA, T. & TAKISHIMA, T. **A Comparative Study of the Metal Miner's Silicosis and Coal Worker's Pneumoconiosis.** *Tohoku J. Exper. Med.* 1961, Mar. 25, v. 73, No. 4, 309–34, 28 figs. [10 refs.]

This paper opens with a short description of the morphology of pneumoconiosis and it is emphasized that dust accumulates more easily in the alveoli related to the alveolar ducts and respiratory bronchioles than in the saccular alveoli. The structure of the walls of the respiratory bronchioles is fragile and thus the mechanical action of dust in coal-miners' pneumoconiosis is so great that it leads to dilatation of the respiratory bronchioles with the development of focal emphysema although generalized emphysematous changes also occur. On the other hand in silicosis it is the chemical action of free silica which predominates and in many cases although nodulation may be widespread large areas of lung tissue remain intact and free of emphysematous changes: and it is silicosis from which the metal-miner suffers. Nevertheless progressive massive fibrosis accompanied by emphysema is not uncommon in silicosis but the latter varies with each case and does not necessarily run parallel to the intensity of silicotic change.

Two cases are quoted, one in a rock driller in a copper mine in whom lung function tests showed little ventilatory impairment although the X-ray category of the lung was Grade III [Category 3 of the International Classification, 1959] and the other was in a coal-miner who showed severe ventilatory impairment but the radiological findings " were far milder than in the former case ".

The complexity of lung function tests is mentioned and a direct writing pressure-volume recorder previously described by two of the authors, Nakamura and Takishima (*Respiration and Circulation*, Japan, 1959, v. 7, 455) is suggested as suitable for routine examination. A study of lung function compared with radiographic grade has been made and the authors consider that in metal-miners obstructive impairment of the lungs is slight even if the radiological grade is P3, and after the age of 40 the lung does not become any stiffer nor does resistance to ventilation increase. Among coal-miners with X-ray grade P1 and P2 in few cases does viscous resistance increase or stiffness of the lung occur unless progressive massive fibrosis develops, but obstructive impairment takes place as the patient gets older and appears rather suddenly after the age of 50.

Ventilatory impairment in silicosis is divided by the authors into 5 main types according to its severity and 70% of the metal-miners were in Groups I and II while only 15% were in Group IV, whereas among the coal-miners there were the same numbers in Groups III and IV as in Groups I and II, or even slightly more in the former two groups than in the latter. Generally speaking progressive massive fibrosis is accompanied by severe ventilatory impairment, but not always; the trend in progressive massive fibrosis is limited to that already noted, thus the greater number of metal-miners are found in Group I while most of the coal-miners were in Group III; however, in metal-miners progressive massive fibrosis is frequently noted in those under 50 years of age while the reverse is true of coal-miners.

After these observations the mechanism of ventilatory impairment is considered in detail and it is concluded that coal-miners' pneumoconiosis takes a longer time to develop than silicosis because of its sheer mechanical action: the effects of hard work, pit dust, humidity, temperature and gas extending over many years and in addition chronic bronchitis, are apt to cause degenerative processes to take place in the lung while the complication of inflammation leads to the development of massive conglomeration, pleural adhesions and chronic emphysema. In silicosis chronic emphysema develops more rapidly especially if complicated tuberculosis is present. Tuberculous foci in the lung affected by free silica may show in the X-ray film in various forms. Progressive massive fibrosis may develop, it may simulate a dust-type of tuberculosis, or it may occur as so-called " soft nodulation ". Compared with metal-miners, complicated tuberculosis occurs only rarely in coal-miners. Clinical features which should suggest the presence of tuberculosis are discussed and treatment is mentioned briefly. The effect of chemotherapy in silicotuberculosis is, however, limited and the disease in metal-miners is exceedingly resistant to drugs.

[The classification of films is not specifically mentioned but presumably it is based on that of the International Labour Office, 1959 (this *Bulletin*, 1960, v. 35, 21). However, in a subsequent paper by two of the authors, published in the same issue (see below) the X-ray classification which has been used is fully described.]

G. F. Keatinge

NAKAMURA, T. & TAKISHIMA, T. **Cardiopulmonary Function Test in Patients with Pneumoconiosis.** *Tohoku J. Exper. Med.* 1961, Mar. 25, v. 73, No. 4, 335–48, 13 figs.

A description is given of the results of spirography determined in various groups of workers by means of a 13·5 L Benedict Roth type of respirometer and a mechanical function test described by the authors (*Respiration and Circulation*, Japan, 1959, v. 7, 455), and carried out by the intraesophageal pressure method combined with a direct writing pressure-volume diagram recorder. Metal-miners, colliers and workers who had a mixed occupational history in both forms of mining, stone cutters, foundry workers and glass workers, were among the groups examined. Static and viscous compliances were low in all groups except the foundry workers whose viscous resistances are in the normal range. In metal-miners the main change of visco-elasticity is restrictive impairment and pulmonary fibrosis occurs only in an early stage, while in coal-miners both restrictive and obstructive impairment are often seen in X-ray categories III and IV and the latter is frequently noted after the age of 50. In stone cutters the findings are similar to those observed in coal-miners.

It is held that pulmonary function tests based on these observations are necessary in patients thought to be suffering from pneumoconiosis. At a primary screening test the MBC is measured and on the results obtained patients are selected for further investigation by the 2-step exercise test which is described. The authors stress the importance of the exercise index and ventilation index combined with oximetry to ascertain severe and

moderate impairment. The 3 fundamental tests to determine compensation are spirography, exercise ventilation and oximetry, and their significance and limitations are discussed.

[The X-ray classification used in this paper appears to be based on, but not identical with, the International Classification, 1959 (this *Bulletin*, 1960, v. 35, 21).]

G. F. Keatinge

ENGELBRECHT, F. M. & BURGER, F. J. **The Influence of Colloidal Silicic Acid on the *in vitro* Oxygen Consumption of Rat Liver Tissue.** *South African J. Lab. & Clin. Med.* 1960, Mar., v. 6, No. 1, 37–42. [18 refs.]

"A study was made of the influence of two CSA preparations, 65% and 92% polymerized, on the oxygen consumption of liver slices, homogenates and cell fractions. Variable results were obtained with the same silica preparation on the respiration of liver slices in different media, and the actions of the two preparations were also different in some media while identical in others. No effect was observed with preparation Y on the respiration rate of liver homogenates, the nuclear fraction and the fluffy layer. CSA had a significant inhibitory action on the oxygen uptake of mitochondria and it is suggested that this may form the basis of the fibrotic changes in silicosis."

ENGELBRECHT, F. M. & BURGER, F. J. **The Toxicity of Silicic Acid.** *South African J. Lab. & Clin. Med.* 1961, Mar., v. 7, No. 1, 16–21. [22 refs.]

In an earlier study the [above] authors suggested that silicotic fibrogenesis might be due to a state of anoxia in the lung induced by the action of silicic acid which harms mitochondria by depressing their oxygen uptake. Although a review of recent work showed that the results obtained by many investigators supported this thesis, nevertheless ROWSELL and LEONARD [*Biochem. J.*, 1957, v. 66, 3P; this *Bulletin*, 1958, v. 33, 1145] were not able to obtain any significant depression of the respiration rates of liver, kidney, spleen, lung and heart slices when they investigated the effects of colloidal silicic acid. This was also the experience of the present authors who however demonstrated the importance of the constituents of the suspension medium, thus silicic acid 92% polymerized has no significant influence while lower polymers of silica (65% polymerized) depress the respiration rate of liver slices significantly.

This view is not universally accepted and accordingly the toxicity of 3 silica preparations, all prepared from an amorphous colloidal silicic acid powder, were studied, namely colloidal silicic acid powder (DC) suspended in 0·5% NaCl solution to give a 55 mM concentration, polymerized silicic acid (DP) prepared from a stock sodium silicate solution (110 mM) diluted with de-ionized water to a final concentration of 55 mM; concentrations of 10 mM, 5 mM and 1·67 mM were obtained by adding to the suspension media specific volumes of the 55 mM solution of DC and DP. Finally monomeric silicic acid (DM) was prepared by adding 55 mM sodium silicate solution directly to the suspension media to give 10 mM, 5 mM and 1·67 mM concentrations. Full details are given of materials and methods used, and the experimental animals were albino rats.

No influence on the oxygen consumption of liver slices in a Krebs-Ringer Phosphate medium was observed *in vitro* over a period of 6 hours at 38°C. with the three silicic acid preparations in different ratios of monomers and dimers to polymers. On the other hand the same silica preparations inhibited the oxygen uptake of liver slices significantly when the slices were pre-incubated with them between 0 and 4°C. before the respiration rates were measured. Preparation DM which consisted of 100% monomers at the beginning of the incubation period but underwent 70% polymerization during this time gave by far the most pronounced inhibition of the respiration rate. It appears that an inverse relationship exists between the degree of polymerization and the toxic effects of the individual silicic acid preparations and although monomeric silicic acid is not harmful at all it becomes so when slightly polymerized. Probably both polymerized silicic acid (DP) and monomeric silicic acid (DM) are able to penetrate the cell membrane, and in order to eliminate the role of the membrane barrier, homogenates were exposed to the influences of polymerized as well as monomeric silicic acid. Oxygen consumption of liver homogenates is depressed by preparations DP and DM (10 mM), more by the former than the latter while preparation DC had no significant influence.

The influence of these three preparations on the respiration of mitochondria was also investigated and it was found that silicic acid DC and DP (10 mM) and DP (1·67 mM) depressed the oxygen uptake of liver mitochondria even without pre-incubation. DM (1·67 mM) did not give a clear-cut effect although on pre-incubation a significant inhibition of respiration was obtained.

The differences in reactions of the various silica preparations may be due to differences in the nature of polymerized silicic acid. Polysilicic acid is formed by the condensation of silicic acid monomers and consists of straight chains, branched chains, networks or highly condensed sphericals depending on the contaminants present. The reactive groups in these molecules are the silanol (–SiOH) groups and if HAZEL's findings (Hazel *et al.*, *J. Amer. Chem. Soc.*, 1949, v. 71, 2256) (that the combining capacity for metal ions decreased as polymerization progressed) is applicable to the reaction of polymerized silicic acid molecules in general it would explain why preparation DC was less toxic than a partly polymerized preparation DP. It seems therefore that silicic acid rather than particles of silica is responsible for the pathological changes in silicosis and that by depressing the oxygen uptake of tissue fractions it causes a state of anoxia in those lung areas where dust particles are localized.

G. F. Keatinge

ENGELBRECHT, F. M. & BURGER, F. J. **The Influence of Silicic Acid on some Respiratory Enzymes.** *South African J. Lab. & Clin. Med.* 1961, Mar., v. 7, No. 1, 22–9. [41 refs.]

In trying to elucidate the mechanism whereby silica produces silicosis many studies have been made of the

action of quartz and other mineral dusts on enzyme activity. Recent literature is reviewed and reference is made to the work of the authors on the depression of oxygen consumption when liver slices, homogenates and mitochondria were incubated with monomeric silicic acid and the latter was allowed to polymerize in contact with these preparations [see above]. The present investigation was planned to determine the influence of polymerized silicic acid on the activity of some respiratory enzymes *in vitro*.

Full details are given of the materials and methods used and the results of the investigation are set out in a series of tables which can be summarized thus: Polymerized silicic acid (10 mM) had no effect on succinic-dehydrogenase, catalase and glutathione, while on peroxidase only slight inhibition of activity occurred. Polymerized silicic acid (10 mM) depressed the activity of cytochrome-oxidase both in its isolated form and in homogenates whether or not aluminium was present. Inhibition was more marked in the absence of aluminium, which therefore protects slightly against the toxic action of silicic acid. The activity of succinic-oxidase, whether isolated or in homogenates, was depressed in the presence of aluminium and in its absence silicic acid enhanced the oxygen consumption of this system in homogenates. Polymerized silicic acid (1·67 mM) inhibited oxygen consumption of cytochrome oxidase in the absence of aluminium. Inhibition was determined by the cytochrome c concentration. The addition of aluminium to the system stimulated oxygen consumption of cytochrome oxidase when the cytochrome c concentration was 1 x 10^{-4}M but depressed it slightly when it was reduced to 0·5 x 10^{-4}M. When aluminium was added to the silicic acid and left to polymerize together before addition to the cytochrome oxidase system containing a high concentration of cytochrome c (1 x 10^{-4}M) a slight inhibition was observed while a significant inhibition of 45·5% was noted when the cytochrome c concentration was 0·5 x 10^{-4}M. When polymerized silicic acid was added separately to the cytochrome oxidase system containing the aluminium and cytochrome c its inhibitory effect was considerably reduced.

The observation that aluminium in small quantities (4 x 10^{-4}M) prevented the cytotoxic action of polymerized silicic acid on the cytochrome oxidase system is interesting in the light of previous findings that small quantities of aluminium when administered simultaneously with silica dust retarded the development of silicotic nodules. The observation that aluminium offered a much better protection of the cytochrome-oxidase system against silicic acid when it was present during the polymerization of the monomeric silicic acid than when aluminium and polymerized silicic acid were added to the medium separately, might indicate that aluminium somehow retards the polymerization of silicic acid by forming a complex or by altering the chemical reactivity of the polymerized silicic acid. Apart from the protective action of small concentrations of aluminium on the cytochrome-oxidase system, preliminary observations during the present investigation indicated that large concentrations of aluminium inhibited the cytochrome-oxidase activity in a similar manner and even to a greater extent than polymerized silicic acid. This dual effect of aluminium on the cytochrome-oxidase system gives a possible explanation of the mechanism whereby small quantities of aluminium prevent silicosis and massive doses produce aluminosis. General cell hypoxia takes place in those lung areas where the dust particles are retained and slowly dissolved and the destruction of macrophages and tissue cells is due to hypoxia and not to activation of a lytic enzyme. Fibroblasts are not affected in the same way perhaps because their metabolism is slower or their metabolic pathways differ from those of other cells; at any rate they survive and proliferate to form the fibrous nodules which encapsulate the particles from which the cytotoxic substances are released. *G. F. Keatinge*

Nicod, J. L. & Reymond, A. Courts empoussiérages et silicose pseudo-tumorale. **[Pseudo-Tumoural Silicosis after a Short Exposure to Dust]** *Schweiz. med. Woch.* 1961, June 24, v. 91, No. 25, 745–7, 4 figs.

In 1946 one of the authors published a note on a case of silicosis occurring in a man of 52 years of age who had only spent about 4 months of his life exposed to risk as a worker in the construction of a tunnel in the Alps. When he died 18 years later massive psuedo-tumours accompanied by nodulation and fibrosis were found in his lungs. In 1954 another case of this kind was encountered: this was a man who had been engaged for about 6 weeks in dry drilling underground in the mountains and similar changes were found in the lungs. Even shorter exposures have been reported by other authors including Lamar who in 1898 recorded the classical case of a worker in quartz mills after only 50 days at risk. Details are now given of another case of the kind.

In 1941 a man then aged 32, who previously had worked either as a labourer or as a stonemason, was employed for about 3 months in tunnelling operations in a very hard black rock. The drilling was done dry and without any form of protection. After that time he went back to his trade as a stonemason and had no further contact with silica but by 1946 he found he was getting short of breath and the next year his capacity for work was estimated at 75%. In 1948 his condition progressed rapidly but despite the occurrence of bronchitis or bronchopneumonia every year he carried on at work until 1959 as a mason during the summer months and as a wood-cutter in winter. There was a history of tuberculosis in the family but numerous sputum examinations during the period 1947–60 failed to reveal the presence of tubercle bacilli and repeated guineapig inoculations were also negative. In 1960 he died in complete cardio-respiratory failure. A post mortem was done and the outstanding findings were the presence of pseudo-tumoral masses in both lungs which were also markedly emphysematous and an enlarged heart chiefly the result of right sided hypertrophy. Despite a careful search no evidence of tuberculosis could be found either on macroscopic or microscopic examination. The lack of evidence of tuberculosis is stressed because the authors are emphatic that pseudo-tumoral masses can develop in its absence, and in support of this view it is indicated that in the case

with an exposure of only 4 months which was reported in 1946 it was equally impossible to demonstrate the presence of tuberculosis. Nevertheless in the worker seen in 1954 who developed silicosis after only 6 weeks' exposure, some evidence of tuberculosis infection was established.

[Silicosis following short exposures to risk has often been reported but the stonemason whose case is described in this paper may have had additional exposure if he was working with sandstone. 60% of massive lesions show no bacteriological or histological evidence of tuberculosis.] *G. F. Keatinge*

BRANDT, A. Der Stand der Silikosebekämpfung in der Deutschen Demokratischen Republik. [**Control of Silicosis in East Germany**] *Ztschr. f.d.g. Hyg. u. ihre Grenzgebiete.* Berlin. 1961, May, v. 7, No. 5, 323–31, 8 figs.

In 1956 a medical and technical organization for the study and control of silicosis was established as part of the Institute of Industrial Hygiene of the Academy in Berlin-Lichtenberg. This organization exercises control from the centre out to the individual works. Every dusty industry, every dusty working place, every silicotic workman and every worker exposed to dust can thus be constantly supervised. For the treatment and prophylaxis of silicosis there are 3 sanatoria: many works have installed prophylactic aerosol plants. The position in East Germany as regards pneumoconiosis is shown by the fact that there were 11,283 cases in 1957 and 12,757 in 1959. This does not indicate any increase in the production of cases because most of them were due to exposure before 1945; the total number of cases appearing after 1945 is distinctly less.

It was not possible to compare the silicosis cases arising since 1945 with the numbers employed in each industry over the whole country but this was done in the Dresden area. It was found that in stone quarrying and in the metal industry (sandblasters and cleaners of castings) the percentage of cases was high, while in copper mining and potteries it was low. The time taken to develop the disease from the beginning of exposure increased from 32·5 years in 1942 to 37 years in 1957. In women this lengthening of exposure time was even more marked, thus showing that the preventive measures are more successful with women.

In most industries the incidence of new cases of pneumoconiosis is falling off; in the metal industry and in stone quarries a more intense effort to control the dust is required. *Charles L. Sutherland*

BRANDT, A. Die individuelle Disposition zur Silikose. [**Individual Susceptibility to Silicosis**] *Ztschr. f.d.g. Hyg. u. ihre Grenzgebiete.* Berlin. 1961, May, v. 7, No. 5, 331–41, 10 figs.

The following conditions are necessary for any investigation of individual susceptibility to silicosis:—

(1) The people investigated must have had an equal exposure to dust;
(2) This exposure must have lasted over 10 years;
(3) The exposure must have led to the development of severe silicosis;
(4) There must be a sufficient number of persons with silicosis to be investigated.

These conditions were found to be fulfilled in the workers at an underground clay mine. Of 117 miners, 80 had silicosis. These were classified according to length of exposure and stage of disease. Whatever the length of exposure, men were found with different stages of silicosis, along with some who had escaped altogether. The fact that under the same working conditions and for the same length of time a worker may develop silicosis grade I, II or III, or may remain completely free, is a satisfactory proof of individual predisposition to the disease.

Figures from the pottery industry show that the time taken for the development of Stages I and III was the same. When men and women workers were compared in this respect, it was found that the time taken in men for the development of individual stages was greater than in women. As women do not work under worse conditions than men, they must be more susceptible. There was also a difference in the effect of entering the dusty work at an older age. In men, rising age of starting work in dust resulted in the development of silicosis in a shorter time. In women, increased age of entry either left the time of development the same or increased it.

The following recommendations are made:—

(1) The amount of dust must be reduced. Even with a moderate reduction more men with a personal resistance will escape.
(2) Those who develop the disease after short exposure where the dust is reduced, must be removed from the work.
(3) Women should only be employed in places where the amount of dust is under the highest permissible normal.
(4) Men over 30 years of age should not be engaged in a dusty occupation. *Charles L. Sutherland*

BRANDT, A. Die Rehabilitation der silikosekranken Arbeiter. [**Rehabilitation of the Silicotic Workman**] *Ztschr. f.d.g. Hyg. u. ihre Grenzgebiete.* Berlin. 1961, May, v. 7, No. 5, 342–6.

The question as to whether removal from dusty work is of any advantage to a silicotic workman is not yet decided. Investigations in clay mine workers and in pottery workers show that patients with silicosis Stage I do not appear to progress to Stage III after removal from dust. Individual patients' however, do progress after short exposure, and it is evident that such workers have a special susceptibility to the disease. The age at entry is of special importance in this connexion since it has been found that older men on entry to dust exposure develop the disease more quickly than younger men. This is not the case with women workers.

In East Germany, the Regulation of November 14th, 1957, prescribes a change of work for notified and compensated individuals. The responsibility for this is placed on the works management. Various difficulties have arisen in carrying out this regulation. One is that

the doctors are not convinced that such a change is always necessary.

Brandt takes the view that those to be removed from dust are those who develop a Stage I after a very short exposure. This short exposure must vary with the type of work but the upper limit must be 15 years. Those with over 15 years' exposure may be considered for removal on account of disability and of age. Workers who have commenced dusty work at an older age must be treated as a special risk. Workers must not be placed in dusty work if they are over 30 years of age.

The change of job must be to one in which the dust concentration is under that laid down as normal in East Germany. By removing the susceptible worker and so raising the general resistance to the disease and by reducing the dust concentration in the air, the fear of silicosis can be eliminated in a reasonable space of time.

Charles L. Sutherland

MILLER, A. A. & RAMSDEN, F. **Carbon Pneumoconiosis.** *Brit. J. Indust. Med.* 1961, Apr., v. 18, No. 2, 103–13, 10 figs. [12 refs.]

A detailed description is given of one case of pneumoconiosis due to carbon black. The subject, a man aged 65 years, had been employed in a rubber factory for 44 years, 21 of which had been continuously spent in the carbon black store where he had been exposed to very heavy concentrations of dust. During his last 11 years employment as a sweeper he was exposed to mixed dust containing carbon black, some talc and small amounts of other dusts.

He had had a cough for 30 years with slight black sputum and occasional nocturnal dyspnoea. From 1956 his symptoms became more marked and X-ray films showed a generalized reticular appearance of both lungs with fibrosis and fibrous masses in the upper zones.

At necropsy the lungs were intensely black. They showed slight generalized emphysema with large apical bullae. The upper lobes were contracted and fibrosed, the pleura thickened and numerous small pneumoconiotic nodules were present. Histological examination showed that the nodules were surrounded by well marked focal and slight general emphysema. The larger nodules consisted of densely pigmented, whorled, hyaline fibres and to some extent resembled silicotic nodules. Vascular changes were conspicuous. No "curious bodies" were seen, nor refractile particles on examination with polarized light, and there was no evidence of tuberculosis.

The dust amounted to 15% of the dried lungs and consisted of 90% carbon and 10% ash. The ash contained 27% of SiO_2 and 10% Fe_2O_3; X-ray diffraction showed that it contained a little mullite and talc with doubtful traces of quartz and cristobalite. Electron microscopy showed that the carbon in the lungs was of two kinds—very fine particles of 20–30 mμ in diameter, similar to furnace and channel blacks, and coarser, 200–400 mμ, similar to thermal black. These were the types of carbon with which the man had worked.

This case is further evidence that disabling pneumoconiosis with massive fibrosis may arise from carbonaceous dust in the virtual absence of quartz [see 2 cases reported by WATSON *et al.*, this *Bulletin*, 1960, v. 35, 856]. The prolonged heavy exposure to carbon black is noteworthy.

A. T. Doig

LISTER, W. B. **Carbon Pneumoconiosis in a Synthetic Graphite Worker.** *Brit. J. Indust. Med.* 1961, Apr., v. 18, No. 2, 114–16, 2 figs.

Only a few cases of pneumoconiosis due to "pure" or "nearly pure" carbon have been reported, and the evidence for such an entity is rather contradictory. This report of one other example is therefore useful. The patient, aged 60, not previously exposed to harmful dust, had been a centre-lathe operator turning and grinding bars of synthetic graphite for 17 years. Chest symptoms started after 13 years at this work. In 1959 he was found to be of moderate physique with a chest expansion of 1½ in. There were no abnormal physical signs in the chest, the blood pressure was 170/90 Hg and the maximum breathing capacity 73 litres per minute. The chest radiograph showed bilateral coarse nodulation, category 3 m. Pulmonary function tests gave findings which were considered compatible with chronic bronchitis with probably only a mild degree of either emphysema or fibrosis.

The graphite had been made from the carbonization of oil or from petroleum coke. A dust sample from the working bench contained less than 0·02% by weight of free silica estimated by chemical methods, but no silica was detected by X-ray diffraction. The ash was 0·45%. Spectrographic examination showed only traces of iron, silicon, magnesium, nickel and calcium. [No mention is made of vanadium.] The particle size ranged from below 0·6 μ to over 100 μ, the median size being 8·5 μ, but this is presumably for deposited dust. 4 other men had worked in the same room for more than 5 years, 2 of whom had chest radiographs taken which were normal.

A comparison is made between this case and others described in the literature.

A. T. Doig

ZAJUSZ, K. & JONEK, J. Wpływ grafitu oraz rożnych mieszanin pyłu antracytowego i krzemowego na płuca białych myszek. [**Effect of Graphite and of Different Mixtures of Anthracene and Silicic Dust on the Lungs of Albino Mice**] *Med. Pracy.* 1961, v. 12, No. 3, 267–79, 10 figs. [19 refs.]

The English summary appended to the paper is as follows:—

"The authors have examined the effect of mice exposure to the inhalation of the following dust mixtures:

"a. pure graphite dust
"b. pure silicic dust
"c. anthracene dust with addition of 0.4 p. c. silica
"d. anthracene dust with addition of 1 p. c. silica
"e. anthracene dust with addition of 2 p. c. silica
"f. anthracene dust with addition of 5 p. c. silica
"g. anthracene dust with addition of 10 p. c. silica.

"In the group of animals to which graphite dust was administered, the prominent activation of dust-absorbing cells was stated, yet any fibrogenic processes were not observed. The fibrous changes were first found in the

group of animals to which anthracite dust with addition of 2 p. c. silica was given. In other groups the fibrous changes are found in a much greater number of animals when the silica dust content was greater.

"When discussing results the authors emphasize the individual sensitiveness of animals to the silica dust, as well as the role of phagocytic elements. At the same time the authors suggest that the silica content overpassing 10 p. c. of total dust content gives results similar to those observed when the pure silica dust is applied."

CRESTI, A. Illustrazione di due casi di siderosi polmonare endogena e di un caso di siderosi esoogena precedentemente interpretati come silicosi. [**Two Illustrative Cases of Endogenous Siderosis and a Case of Exogenous Siderosis previously regarded as Silicosis**] *Acta Med. Legalis et Socialis.* Liège. 1959, July–Dec., v. 12, Nos. 3/4, 119–29, 5 figs. [15 refs.]

The two cases of endogenous pulmonary siderosis (haemosiderosis) occurred in men who had worked for long periods of time in mercury mines and had been accepted as suffering from silicosis. Both men died with clinical evidence of heart failure and autopsy showed that in one of them the heart was obviously enlarged and signs of fibrous endocarditis were present in the mitral and tricuspid valves, but above all there were extensive changes in the aortic semilunar cusps which were completely fused together, contracted and deformed, and almost entirely calcified or ossified, giving a picture of aortic stenosis. In the other man mitral stenosis was found on post-mortem examination and in neither case was there any evidence of silicosis. The case of exogenous pulmonary siderosis occurred in a man who had suffered from pneumonia on 3 occasions in 1950, 1953, and 1954 and from 1942 to 1954 had worked for 4 years in the actual making of glass where he was not exposed to any materials containing silica, and from 1946 onwards as a polisher. This work involved the use of ferrous oxide but there was absolutely no exposure to silicon dioxide. When he sought acceptance as a case of silicosis, X-ray examination showed the whole pulmonary network to be greatly exaggerated with hilar shadows associated with diffuse micro-nodulation. On the basis of these findings it was stated in the X-ray report that the condition was one of severe pulmonary silicosis with a tendency towards pseudo-tumoral changes. Nevertheless he was rejected as a case of silicosis on the ground that the occupational history without any possibility of doubt excluded a diagnosis of silicosis, and made that of pulmonary siderosis quite certain.

These 3 cases have been reported because they all show the importance of a proper history, and in addition the first demonstrates the fact that when aortic stenosis is associated with disease of the mitral valve, haemosiderosis may occur. The majority of authorities hold that it is possible to make a differential diagnosis between silicosis, siderosis and endogenous haemosiderosis by means of the different X-ray appearances of the various conditions, such for example as the size of the nodules, their distribution, whether symmetrical or otherwise, the degree of hilar involvement, the extent to which emphysema is present and so on, but the author from an experience of the interpretation of many hundreds of X-ray films of miners thinks that a diagnosis should not be based on X-ray findings alone: clinical examination and an occupational history are essential. Biopsy by lung puncture has already been the object of criticism and even if heart failure cells are found, their presence does not exclude the possibility of concomitant silicosis.

[Excellent sentiments with which no experienced observer will disagree.] *G. F. Keatinge*

HIGGINS, I. T. T. **Bronchitis.** *Trans. Ass. Indust. Med. Officers.* 1961, Jan., v. 10, No. 4, 118–25, 11 figs. [Numerous refs.]

In England and Wales over 25,000 deaths from chronic bronchitis are certified annually, and 25 million working days lost. At ages 55–64 the mortality is six times higher in men than in women, and 40 times higher than it is in the United States and Scandinavia. In London the mortality is highest towards the east and north, where, owing to the prevailing winds, air pollution is maximal. Mortality rates for both men and women are approximately twice as high in conurbations as in country areas. Correlations have been found between mortality from bronchitis and atmospheric deposits, SO_2 content, and smoking habits.

In a study of postmen, wastage rates due to premature retirement or death from bronchitis and pneumonia were found to be concentrated in the great towns and industrial areas, and were correlated with thick polluted fogs, but not with population density or domestic overcrowding; this suggests that social factors are not of prime importance. A close association has also been found between clinical illnesses in patients with chronic bronchitis and smoke concentration.

During the past 6 years representative samples of the population have been studied in 3 industrial and 2 rural areas, for prevalence of bronchitis—arbitrarily defined by persistent sputum for at least 3 months of the year and one or more chest illnesses per year. The curve of incidence for men aged 55–64 rose progressively in the following groups—rural workers, foundry work, urban non-miners, coke oven workers, miners, furnace men, and chemical workers. A higher prevalence of respiratory symptoms, especially persistent cough and sputum, was found in smokers than in ex-smokers and non-smokers, particularly cigarette smokers, and the same was true for measurements of ventilatory capacity.

As regards infection, the lower respiratory tract in bronchitis is probably colonized from the upper respiratory tract, mainly with pneumococci and *Haemophilus influenzae*. This change seems to be precipitated by certain virus infections, notably influenza, the common cold, and the adenoviruses, and possibly also is expedited by the irritation of fog or fumes and gases. Certain constitutional and genetic factors may also have an influence.

From all these data a picture of the pathogenesis of bronchitis is emerging. It is that of a vicious circle starting with irritation of the mucosa from infection or air pollution producing hypersecretion of mucus and leading to infection of the bronchi, further mucus secretion and respiratory obstruction with all the sequelae of

destruction of tissue, emphysema, bronchopneumonia, bronchiectasis, abscess formation, and obliterative fibrosis.
H. Stanley Banks

FERIN, J. **Elimination of Dust from the Lungs and the Influence of the Reticuloendothelial System.** *Ann. Occup. Hyg.* London. 1961, Jan., v. 3, No. 1, 1–5, 1 fig.

Dust which reaches the respiratory bronchioles, alveolar ducts or alveoli is beyond the reach of the ciliated epithelium. Phagocytosis is believed to be a means of elimination of such dust. Ferin finds that the administration of silica particles by a single intratracheal injection has a stimulating effect upon the reticuloendothelial system [RES] in general as judged by the absorptive capacity of the liver for intravenously administered colloidal silver. In a further experiment, trypan blue was injected to stimulate the RES a few days before the animals were exposed to dust. This led to a much greater elimination of dust injected into the lungs as compared with controls. It is concluded therefore that the functional state of the RES is closely connected with the elimination of dust from the lungs. *J. Gough*

CREMER, Jill E. **The Toxicity of Tetraethyl Lead and Related Alkyl Metallic Compounds.** *Ann. Occup. Hyg.* London. 1961, June, v. 3, No. 4, 226–30, 1 fig.

This paper shows how biochemical studies were used in elucidating the mechanism of tetraethyl Pb poisoning. Some of the work was reported before [this *Bulletin*, 1960, v. 35, 112]. Other alkyl compounds were investigated *in vivo* and a comparison made with *in vitro* results. The latter were mostly based on oxygen consumption and lactic acid levels when rat brain slices were incubated in a Krebs-Ringer phosphate medium with a glucose substrate.

Rat LD50 (parenteral) in mgm. per kgm. were: tetra- and tri-ethyl Pb respectively 15·4 and 11·2; tetra- and tri-methyl Pb 105 and 25–30; tetra- and tri-propyl Pb 200 and 20–30.

Diethyl Pb and Pb acetate were relatively non-toxic and had little effect on brain slice metabolism. Triethyl Pb, the most toxic *in vivo*, had the greatest biochemical effect but tetraethyl Pb was without effect *in vitro* though both compounds, in only slightly differing doses, caused identical poisoning—tremors and convulsions preceding death. However, the activity of brain slices from rats killed 4 hours after injection of 20 mgm. tetraethyl Pb per kgm. showed lowered oxygen consumption and increased lactic acid identical to that in rats injected with 10 mgm. triethyl Pb per kgm. (After diethyl Pb or Pb acetate, 40 or 100 mgm. per kgm. there was virtually no change.)

Tissue distribution of triethyl Pb after injection of tetraethyl Pb was considerable, and the pattern similar to that after injection of triethyl Pb. The site of conversion was found to be the liver. When rat liver was incubated in the medium containing tetraethyl Pb, there was linear production of triethyl Pb, 60 μgm. per gm. wet weight per hour. This did not happen when brain or kidney were similary treated.

With high enough doses the methyl compounds caused poisoning similar to that of the 2 ethyl compounds, but with the propyl compounds tremors or convulsions were absent, rats remaining quiet and weak until death. With triethyl tin a different response occurred. Yet the altered glucose metabolism was the same in brain slices from animals poisoned by the 4 tri-compounds. This shows the need for finding a more specific biochemical lesion in nerve tissue. *M. Patricia Fitzsimons*

KARPATKIN, S. **Lead Poisoning after taking Pb Acetate with Suicidal Intent. Report of a Case with a Discussion of the Mechanism of Anemia.** *Arch. Environment. Health.* Chicago. 1961, June, v. 2, No. 6, 679–84, 1 fig. [20 refs.]

The literature reports 7 cases of acute Pb poisoning from ingestion of large amounts in the last 20 years; 6 of these subjects had anaemia. Data are also given of the progressive anaemia reported after intravenous Pb treatment in cancer, which regenerates quickly. The aetiological theories that have been advanced for Pb anaemia from large amounts or from repeated small amounts are surveyed, and suggest the soundness of the hypothesis that Pb restricts protoporphyrin formation—rather than preventing its utilization—but yet the basis for the increased excretion of coproporphyrin III remains to be explained.

A woman, aged 37, an alcoholic who had had jaundice, began vomiting 2 hours after drinking a lead and opium solution, 7 gm. Pb acetate. She had pyrexia, a large liver, haemorrhoids, oedema of legs and albuminuria. She soon developed pain in ankles and hands with transient extensor hand paresis, and later generalized weakness, occipital headache for 5 days and paraesthesiae. Albuminuria had ceased. Recovery was uneventful.

Meantime, when tested in glassware washed in nitric acid, blood and urine showed Pb levels within upper normal ranges; otherwise the highest blood value was 0·228 mgm. per 100 gm. on the 8th day. When provoked by $CaNa_2$ EDTA—2 and 4 gm. intravenously on the 18th and 19th days—urinary excretion increased markedly to 4·9 and 3·1 mgm. per 24 hours. Calcium gluconate had been given earlier.

By the end of the first 2 weeks the haematocrit had fallen steadily from 54 to 34, haemoglobin from 15 to 10 gm. and red cells from 5·96 to 3·72 million, the lowest values. Serum iron was 57 μgm.%, the iron-binding capacity 280 μgm.%. The urinary coproporphyrin III tests were still positive on the 19th day and then became negative. Haemoglobin electrophoresis, red cell survival time and fragility, reticulocytosis (0–2%), other investigations and many liver function tests were normal, as were routine clinical investigations and the blood urea. The haematocrit reached 41 on the 25th day, haemoglobin 12 gm. *M. Patricia Fitzsimons*

RADOSEVIC, Z., ŠARIC, M., BERITIC, T. & KNEZEVIC, J. **The Kidney in Lead Poisoning.** *Brit. J. Indust. Med.* 1961, July, v. 18, No. 3, 222–30, 2 figs. [52 refs.]

Investigations of renal function were made in 53 patients with Pb poisoning who were under observation

in Zagreb for 5 years in order to obtain more evidence about the effect of Pb on the kidney. Different opinions that are held on the question whether Pb damages the kidney, and if so how, are reported. It is concluded that Pb can produce renal damage, that this may be functional and varied or, if exposure is long, anatomical, and that the phase of the disease at which tests of function are made is important. Changes shown by different tests did not occur simultaneously or at the same rate of progress. This may account for conflicting reports.

Tests, apart from laboratory criteria of Pb poisoning, include the urine concentration test, the phenol red test, urea clearance, blood urea, blood pressure and ordinary urine examination. The only 2 subjects with chronic progressive nephropathy with transition to nephrosclerosis and uraemia, had been exposed the longest, 22 and 35 years; the patients were aged about 50 and 45 and had had poisoning repeatedly. Kidney microscopy and tissue Pb contents are given in one case. Apart from these 2 eventually fatal cases, only 1 other patient had permanent hypertension—8 years' exposure, repeated colic, changing tests—and only 1 other had abnormalities in the urine during pleurisy. In 23 patients various functional changes were found which tended to become normal without any urinary abnormality. The data supported the explanation that the partial impairment of renal function is brought about by disordered intrarenal circulation resulting from the spastic effect of Pb on intrarenal blood vessels, and direct toxic or indirect hypoxic effect of Pb on the tubules.

Most of the patients were aged 20 to 45 years; length of exposure was from 2 months to 14 years, except for 9 in which it was unknown and the 2 above; 40 men and 4 women had occupational Pb poisoning, and 5 men and 4 women used lead-glazed pottery for food and drink, as did 2 of the 40 men. The illness was gastrointestinal in type in 49 patients and neuromuscular in 3, and the other had encephalopathy and gastrointestinal symptoms. *M. Patricia Fitzsimons*

BRUUSGAARD, A. Vurderingen av blyverdier i urin. **[Evaluation of Lead Values in Urine Spot Samples]** *Nordisk Hyg. Tidskr.* 1961, v. 42, Nos. 3/4, 71–4, 1 fig. English summary (6 lines).

In determinations of lead content carried out on 779 samples of urine from persons with occupational exposure a positive correlation could be demonstrated between lead content and the specific gravity of the urine. The author accordingly suggests that the observed lead content should be adjusted to a value corresponding to a standard specific gravity of 1025 by multiplying by 25 and dividing by the last 2 figures of the specific gravity of the sample. A lead content of 90 μgm. per litre in a sample with a specific gravity of 1010 thus becomes 225 μgm. per litre when corrected, indicating a marked degree of exposure. *D. J. Bauer*

BEAVER, D. L. **The Ultrastructure of the Kidney in Lead Intoxication with particular reference to Intranuclear Inclusions.** *Amer. J. Path.* 1961, Aug., v. 39, No. 2, 195–208, 11 figs. on 6 pls. [51 refs.]

ZIELHUIS, R. L. Maximal zulässige Grenzwerte biologischer Reaktionen in der Verhütung gewerblicher Bleivergiftungen. **[Maximal permissible Limits in Biological Reactions Relating to the Prevention of Occupational Lead Poisoning]** *Zent. f. Arbeitsmed. u. Arbeitsschutz.* 1961, June, v. 11, No. 6, 129–34.

The purpose of the work described in this paper was to formulate the limits of such biological reactions as stippling of blood cells and urinary coproporphyrin concentration above which lead poisoning could be presumed to be occurring.

These biological reactions were measured, by methods which are detailed, in repeated examination of 39 workers in lead over a period of 18 months, and single examinations of 500 men working in a great variety of industries in which lead is used.

There is a full account of the findings in these examinations with detailed tables.

In the summary it is stated that it should be the aim of those concerned in the medical supervision of lead workers to prevent a lessening of the haemoglobin concentration. A prognostic indication of lead poisoning may be an increase in stippled cells and the maximal limit of these is set at 1 per 1,000 erythrocytes. Urinary coproporphyrin was determined by the method of DONATH (*Arhk. Hig. Rada.*, 1956, v. 7, 77) which, however, is too sensitive in that a maximum limit cannot be set for individual use. The method is, however, suitable for determining the average concentration of urinary coproporphyrin in groups of men and there was found to be a constant correlation between this average coproporphyrin value and the haemoglobin level. An average urinary coproporphyrin figure corresponding to Stage 2–2·5 on Donath's scale (Stage 2=50–100 μgm. per litre and Stage 3 =100–200 μgm. per litre) may be taken as a maximal limit for groups of workers at risk of lead poisoning.

The maximum allowable concentration of lead in the atmosphere should be about 0·1 mgm. per cubic metre of air. *M. E. Delafield*

KING, E. & THOMPSON, A. R. **The Measurement of Lead Absorption in Industry.** *Ann. Occup. Hyg.* London. 1961, June, v. 3, No. 4, 247–63, 14 figs.

This paper is based, in part, on data originally published in *Industrial Medicine and Surgery*, and reviewed in this *Bulletin*, 1961, v. 36, 333.

LEVEQUE, J., TROTEMANN, S. & MARTI-FECED, C. Étude de l'élimination de base de la déhydroépiandrostérone chez le manganique. **[Investigation of Dehydroandrosterone Urinary Excretion in Subjects with Manganism]** *Maroc Méd.* 1961, Apr., v. 40, No. 431, 389–91.

During investigations of urinary steroids in manganese poisoning determinations were made of dehydroandrosterone (DHA) and neutral 17-ketosteroids. Excretion of the latter had been found to be diminished in manganese

workers and at periodic examinations a number of men complained of sexual disabilities. No clear relationship had been found between impotence and total urinary 17-ketosteroids, one-third of which are of testicular origin, and a study was made of the origin—testicular or suprarenal—of the hormones and metabolites which are described.

18 miners, with more or less long-standing manganese poisoning, who came from various regions of Morocco, were admitted to hospital for collection of 24-hour specimens of urine. Daily excretion was checked by measuring creatinine levels. The mean excretion of 17-ketosteroids was 8·19 mgm. per 24-hours (normal 12–13, with a range of 10–15) and of DHA was 0·608 mgm. per 24-hours (normal 1·55). The table of results shows 3 men with normal values, means 10·97 and 1·49 respectively. Mean values of the remaining 15 were 7·64 and 0·43, decreases of 40% and 72% of normal. In these 15, DHA was 5·6% of 17-ketosteroids and in the other 3, 13·4% (normal 12%–13%).

The degree of decrease in DHA values was surprising and points to suprarenal injury. This has been investigated and results are being published.

M. Patricia Fitzsimons

BROWNING, Ethel. **Toxicity of Industrial Metals.**

This book was reviewed on p. 958.

METZNER, F. N. & LIEBEN, J. **Respiratory Disease associated with Beryllium Refining and Alloy Fabrication. A Case Study.** *J. Occup. Med.* Chicago. 1961, July, v. 3, No. 7, 341–5. [16 refs.]

This paper is a sequel to papers by Lieben and his co-workers in 1959 [this *Bulletin*, 1960, v. 35, 762]. Further follow-up of the workers from a large beryllium refining plant in Pennsylvania and of the nearby community has added 10 new cases of chronic beryllium poisoning to the 58 already known. It is also estimated that 280 cases of acute chemical pneumonitis and tracheobronchitis have occurred over the past 20 years. The local hospital post-mortem records reveal 3 cases of carcinoma of the lung in persons exposed to beryllium for 10–15 years. The pathologist reported no evidence of chronic pulmonary beryllium disease in these cases. 6 cases of sarcoid disease were discovered in the area, which was thought not to exceed the incidence reported by others.

A greater number of cases among females than males is reported and in 3 instances there have been 2 cases in one family. Of 12 cases of acute chemical pneumonitis or tracheobronchitis occurring during 1957–1959 "two or three" have residual pulmonary abnormalities.

It is reported that the plant has taken the steps necessary to prevent the further development of beryllium disease.

C. H. Wood

GERARDE, Horace W. [M.D., Ph.D.] **Toxicology and Biochemistry of Aromatic Hydrocarbons.** pp. xiii + 329, 89 figs. 1960. Amsterdam. London. New York. Princeton: Elsevier Publishing Co. [30s.]

The author's purpose was to assemble information from scattered sources for those who are concerned with the prevention, detection and treatment of exposure to the aromatic hydrocarbons of industrial importance. It has been achieved in a remarkable manner. The value of the book for the physician, industrial hygienist and toxicologist, and also for management and for those people who are trying to learn something about applied science, is enhanced by the readable way in which difficult scientific matter is presented, some of it not published before. This surely comes from membership of the working environment team and carries the mark of the doctor versed in organizational communication, that is to say the industrial medical officer. The author is toxicologist to the Medical Research Division, Esso Research and Engineering Company, Linden, New Jersey, the editor, Dr. Ethel BROWNING.

After reading this handy, well-printed book, it was of interest to note—and endorse—the publisher's description, "... an exhaustive yet compact and easily read account of the present state of knowledge on this subject ... [and] original information." This has been achieved because numerous cross references are so clearly and neatly dovetailed that repetition is unnecessary and because groups of structural formulae, diagrams, photographs, graphs and 59 easily seen tables collect facts together, making the text a description and discussion that flows along. The substance is drawn from the world literature to the end of September 1959 and the author's own research, with particular attention given to recent work from various sources not yet in books. The high standard of his published work is carried over into hitherto unpublished data.

General considerations appear in the first 5 chapters: definitions and basic chemistry; nomenclature and classification; sources and uses; physical properties and analytical procedures; toxicological effects on senses, mucous membranes, subcutaneous tissue, eye and skin; systemic intoxication, acute and chronic, and the relationship between chemical constitution and toxicity; biochemistry of absorption, distribution, excretion and metabolism; and methods of prevention, detection and treatment.

Under similar headings 20 important industrial aromatic hydrocarbons are then dealt with in detail, with reference back to, for example, tables of toxicity data. A chapter follows on the polycyclic aromatic hydrocarbons with a discussion on carcinogenesis. Chapter 13 describes mixtures, the solvents, lubricants and fuels. The glossary, bibliography and index round off a truly useful monograph.

Threshold limits as recommended by the American Conference of Governmental Industrial Hygienists are given when existing, and when not tentative values are based on analogy. The impossibility of applying working-day limits to atmospheric pollution is explained and reference is made to the low limits established by the U.S.S.R. The relevant part of Imperial Chemical Industries' more comprehensive table of toxic concentrations is reproduced from the 1952 edition. Periodic health examinations are necessary with almost all compounds mentioned, and these in Italy are required by law.

Treatment for gassing accidents and for eye or skin contact is followed by a section which considers ingestion and aspiration together because of the importance of

minimizing the latter, especially in children who have swallowed kerosene. Toxicity by mouth is relatively low but in the lung high. "Extensive pulmonary edema and hemorrhage follow the aspiration of a fraction of a milliliter of the liquid aromatic hydrocarbons" because the low surface tension allows the irritant liquid to spread over a large area. Therefore gastric lavage is contraindicated, except possibly for "enormous doses". Vegetable or animal fat is safe to administer and effective. In rats olive oil tends to diminish absorption of aromatic hydrocarbons. Mineral oil is not safe because, by causing rapid passage along the intestinal tract and thereby increasing the absorptive area, it increases the rate of absorption, and high blood levels of these compounds cause extensive endothelial damage.

"The worker is a continuous biological sampling device whose blood stream is separated from the air he breathes by two endothelial membranes a fraction of a micron in thickness. The pulmonary capillary and alveolar endothelium are readily permeable to vapors and mists containing chemicals . . ." Detection of chemical or a metabolite in tissues or biological fluids may serve as a test for exposure more convincingly than measurement of atmospheric concentrations. This does not necessarily mean injury. The distinction between toxicity and hazard is made clear. The urinary sulphate ratio is given for some 60 aromatic hydrocarbons and its significance discussed. When there is no established test for exposure or incipient toxicity this is discussed in relation to other compounds or the need for further research. There is no enzymatic or biochemical test for the detection of incipient or latent toxicity. Benzene, unquestionably the most dangerous aromatic hydrocarbon, and toluene are the only aromatic hydrocarbons for which correlations have been made between air concentrations and the amounts of urinary metabolites excreted during working conditions. *M. Patricia Fitzsimons*

DAWBORN, J. K., RALSTON, Mary & WEIDEN, S. **Acute Carbon Tetrachloride Poisoning. Transaminase and Biopsy Studies.** *Brit. Med. J.* 1961, Aug. 19, 493–4, 1 graph & 3 figs. on pl. [12 refs.]

Serial studies in a case of ingestion of CCl_4 show, among other things, the association of the levels of serum glutamic oxalacetic acid with the degree and the course of liver damage and regeneration.

The patient, aged 22, vomited 2 hours after accidentally swallowing a mouthful of CCl_4 and passed dark urine. Next day anorexia and abdominal pain increased. When he was admitted to hospital on the 3rd day the epigastrium was rigid and tender, the urine contained granular casts and albumin and several biochemical analyses showed abnormalities. On the 5th day the patient felt well and clinical recovery was complete on the 10th.

Choline and antihistamine treatment, which lessens damage to mitochondria, may have contributed to the prompt fall of SGOT levels, from 2,120 units on the 3rd day to 520 on the 5th, by lessening the release of enzymes from the liver cells. A full diet was given with nicotinic acid and vitamin K. The prothrombin index, initially 19% of normal, was 100% on the 9th day, bromsulphthalein retention was 38%, and serum amylase had fallen from 266 units to below 100, urinary diastase from 80 to 20 units. Biochemical findings at this time became normal. Renal biopsy performed on the 10th day showed only pigment in epithelium of medullary tubules, and gastric biopsy, 11th day, was normal.

Liver biopsies are illustrated. On the 9th day centrilobular necrosis affected one-third to one-half of every lobule in the specimen—an image related to the initial SGOT level of at least 2,120 units, though now normal—and there was infiltration with lymphocytes, plasma cells and macrophages containing bile pigment. However, the reticulin network was intact and surviving cells were seen near the central vein. On the 28th day biopsy still showed centrilobular damage and fatty changes, but large regenerating cells were seen. On the 70th day, apart from a few inflammatory cells in sinusoids and some fatty changes, histological appearances were normal.

[See this *Bulletin*, 1961, v. 36, 995.]

M. Patricia Fitzsimons

TÖPPICH-STRAUB, D. & MINDEN, H. Serumfermentbestimmungen bei Tetrachlorkohlenstoffvergiftung. **[Estimation of Serum Enzymes in Carbon Tetrachloride Poisoning]** *Arch. f. Gewerbepath. u. Gewerbehyg.* 1961, v. 18, No. 4, 442–57, 5 figs. [21 refs.]

Carbon tetrachloride exerts its toxic action on the liver and kidneys by disrupting enzymes, penetrating into the walls of the hepatic cells, attacking the mitochondria and inhibiting the synthesis of pyridine nucleotide by disturbance of the tricarboxylic cycle.

Biochemical investigations in animals with acute carbon tetrachloride poisoning have shown that the primary injury of the mitochondria leads to an increase in activity of glycolysis and probably also of the adenosine phosphate enzymes, with diminution of energy-rich phosphates, disturbance of formation of co-enzyme A and diphosphopyridine nucleotide and of the ferment reactions dependent on these.

The presence in the serum of transaminases (serum glutamate oxalacetate—SGOT, and serum glutamate pyruvate—SGPT) and of aldolase and cholinesterase, in many liver diseases may be explained partly by their extravasation from the necrotic liver cells, partly also by a compensatory increase from the non-injured cells.

The authors have investigated the behaviour of these enzymes in animals with subacute carbon tetrachloride poisoning. All, with the exception of cholinesterase, were raised, with a maximum on the third day.

With SGOT and SGPT the peak rise on the third day was from an initial level of 18·6 to 181·2 units and from 48·3 to 418·1 units respectively. Serum proteins, estimated by paper electrophoresis, showed as their chief variation an increase in gamma globulin on the 13th day, with a simultaneous decrease in albumin.

The blood picture showed only a slight decrease in haemoglobin; loss of weight reached its maximum on the 13th day; amino acids in the urine showed no significant variation and there was no albuminuria. Histological examination showed no changes in the kidneys and only slight fatty infiltration in the liver.

It is concluded that from the clinical and diagnostic point of view estimations of transaminases and aldolase are valuable if carried out during the first few days. Their rapid subsequent fall is explained by a disturbance of ferment synthesis rather than by active regeneration, which cannot be regarded in carbon tetrachloride poisoning, as it can in inflammatory diseases of the liver, as a certain sign of return to normal function. The prognosis is, however, on the whole good, owing to the tendency of the liver to regenerate. *Ethel Browning*

YAMAMOTO, A. **Changes in the Swelling Phenomenon of Mitochondria and in Lipides in the Mitochondrial Fraction of the Liver following Carbon Tetrachloride Poisoning.** *Med. J. Osaka Univ.* 1960, Dec., v. 11, No. 3, 265–77, 3 figs. [29 refs.]

KÄHLER, H. J. & MERKER, H. Chronisch myeloische Leukämie nach langjährigem Kontakt mit Benzol. [**A Case of Chronic Myeloid Leukaemia after Prolonged Exposure to Benzene**] *Deut. med. Woch.* 1961, June 9, v. 86, No. 23, 1135–40, 1163, 4 figs. [19 refs.]

A discussion of the various factors which may be important in the causation of leukaemia introduces this account of a case of myeloid leukaemia in a workman aged 57 who had been exposed to inhalation of benzene in the course of his work for 7¾ years.

After about 2 years of this work he suffered from dreams in which people and landscapes were coloured yellow and red, headaches and sleepiness. He continued at his work. It was not until about 6 years later at a routine medical examination—and such examinations had been carried out regularly at the factory where he worked—that a leucocyte count of 23,000 and other abnormalities in the blood picture were found. The original complaints had got somewhat worse but there were no other significant symptoms.

In spite of changing his work and the treatment the disease has got progressively worse. There is a very detailed clinical account of the case with tables and figures.

There is a careful discussion in which it is stated that a relationship between exposure to benzene and myeloid leukaemia must be assumed. It is suggested that a determination of the alkaline phosphatase activity of leucocytes should be added to the routine medical examination which is not adequate for the discovery of early blood changes, as this case has shown. It is recommended that work in occupations where there is the danger of exposure to benzene should not continue for periods exceeding 2 years. *M. E. Delafield*

SOUCEK, B. Retention und Ausscheidung von Methylchlorid und Tetrachlorkohlenstoff bei Ratten. [**Retention and Excretion of Methyl Chloride and Carbon Tetrachloride in Rats**] *Arch. f. Gewerbepath. u. Gewerbehyg.* 1961, v. 18, No. 4, 370–83, 1 fig. [24 refs.]

The author describes investigations into the amount of retention and speed of excretion of methyl chloride in white rats, after subcutaneous injection of 330 μgm. or 1,200 μgm. of methyl chloride in the form of an aqueous solution containing 660 μgm. per ml. The amount expired was estimated with the lower dosage and the free methyl chloride in 24-hour samples of urine and faeces, and the amount in the blood, with the higher dosage.

The average amount expired was 27% of the dose, very rapidly reaching its maximum in 40 minutes and being complete in 2 hours. Amounts in the urine and faeces were so small as to be unmeasurable. In the blood, a maximum of 1·4% was found within 2 minutes of injection—about 10 to 100 times more than with chloroform or carbon tetrachloride. It disappeared rapidly; after 20 to 25 minutes it was imperceptible. It is concluded that about 70% of the administered dose is metabolized within 20 to 30 minutes, and that, owing to this fact and that it is much less soluble in fat than chloroform or carbon tetrachloride, it is unlikely to be deposited in fatty tissues.

Previous experiments [this *Bulletin*, 1952, v. 27, 70] demonstrated about the same amount of carbon tetrachloride in the liver, brain, kidneys, muscles as in the blood, and about one-half this amount in the lungs, spleen and heart muscle, 3 times as much in the bone marrow, and 8 times as much in fatty tissue.

The author's investigation on the excretion and retention of carbon tetrachloride was carried out by subcutaneous injection into white rats of a solution in olive oil in amounts varying in one group from 43 to 410 mgm. per kgm., and in another from 1,450 to 3,650 mgm. per kgm. Urinary and faecal excretion in 24 hours in both groups was either absent, or only trace amounts were found. In the blood one hour after injection the amounts were very small—0·005 to 0·01% of the dose. The red corpuscles retained about 2·5 times as much as the plasma.

Elimination is almost entirely by the lungs—an average of 98% unchanged. The speed of expiration is about the same as that of trichloroethylene and 2·5 times as rapid as that of chloroform.

The liver, lungs and brain contained about the same amount as the blood, the fatty tissues 20 times as much. *Ethel Browning*

HOTTA, K. **Experimental Studies on Carbon Disulfide Poisoning. Part I. Experimental Studies on the Adrenocortical Function of Rabbits poisoned with Carbon Disulfide.** *Nagoya J. Med. Sci.* 1961, Mar., v. 23, No. 4, 431–45, 6 figs. [26 refs.] **Part II. Experimental Study on Adrenocortical and Liver Function and Lipemia Clearing Factor in Chronic Carbon Disulfide Poisoning.** *Ibid.*, 445–60, 10 figs. [31 refs.]

This work was undertaken to see whether the picture of mild CS_2 poisoning depends on a specific or non-specific adrenal response. The pattern of adrenal function was examined in relation to liver function and possible arteriosclerotic changes. The results are shown in many curves and are discussed in much detail.

Part I. Daily measurements for 20 days were made of urinary excretion of adrenal hormones, glucuronic acid and creatinine in female rabbits injected intramuscularly

once with 0·4, 0·7 or 1·4 ml. CS_2 or daily for 7 days with 0·4 ml. CS_2 or with equivalent volumes of the vehicle, olive oil (the controls). The hormones were 17-hydroxy corticosterone, 17-ketosteroids and chemicosteroids. Tables show values found in normal rabbits for these and additional investigations done in Part II.

The single injections were followed by hormone excretion curves similar in pattern to those from other forms of stress. The duration of early increased excretion was longer with the larger doses, and with the largest dose there was an initial short shock-phase decrease. During the repeated injections, after a transient peak, values fell to normal and afterwards fell lower before returning to normal: since no sign of an adaptive reaction, a rising curve, followed the alarm reaction this was considered to be due to a direct effect on the adrenal cortex. Creatinine excretion had a similar pattern though with the largest dose the return to normal was delayed. The 4 glucuronic acid curves differed: 0·4 ml. caused a brief rise, 0·7 ml. a 2-peak rise and 1·4 ml. a fall followed by a rise; the repeated injections caused an increase which was maintained. This probably reflected an alarm reaction and also an accelerated synthesis in the liver.

Part II. Serial measurements were made—as in Part I and of other substances—for 115 days from female rabbits injected on alternate days with 0·1 or 0·2 ml. per kgm. CS_2 or with olive oil. The curves of substances mentioned above showed similar patterns, as did the curve of plasma mucopolysaccharides—at first a rise and fall to normal and then during the second half of the period a fall below control values, though creatinine values remained slightly above control values. In addition, body weight fell steadily for 55 days, liver function as shown by the cephalin-cholesterol flocculation (CCF) test and urobilinogen excretion decreased during the second half and serum glycoprotein increased to a plateau in 30 days. The terminal lipoprotein lipase content of the aorta was lower than that of controls, and adrenals, liver, kidneys and some aortae showed degenerative changes. No changes were seen in coronary arteries.

The early excretion increases were considered characteristic of a systemic response to a stressor and the late decreases were probably due to a specific action of CS_2 on the adrenal cortex and the latter was paralleled by liver dysfunction. The decreased lipaemia-clearing ability was conjectured as showing an effect preliminary to arteriosclerotic changes. *M. Patricia Fitzsimons*

WANG, C. C. & IRONS, G. V., Jr. **Acute Gasoline Intoxication.** *Arch. Environment. Health.* Chicago. 1961, June, v. 2, No. 6, 714–16.

This case report, another example of disregard of safety rules by a man in a hurry, illustrates the rapidity of the lethal effect from exposure to petrol vapour; familiarity with this vapour has lessened appreciation of potential danger. An aircraft mechanic, aged 25, knew the rules and was in charge of a team whose job it was to change a pump inside the wing tank. He sent 1 of his 2 helpers for the airblower to clear the tank of vapour. Clearing would take 2 hours, changing the pump only 15 minutes. Meanwhile the other sentinel went inside the aircraft to bandage a cut finger, and the mechanic nevertheless started the job. He was found unconscious 5 minutes later and was removed for immediate artificial respiration. After several weak uncoordinated gasps he died. There was acute pulmonary oedema and exudative tracheobronchitis; congestion of liver and spleen; and haemorrhagic pancreatitis, a manifestation of vascular endothelium damage. The tissue lactic acid was 154 mgm.%. An unidentified volatile material was extracted from the brain by steam distillation.

Similar tanks, after being emptied of aviation petrol and before being cleared by jets of steam, contained 0·5%–1·6% of petrol vapour. In animals 1% causes convulsions and 1·6% immobility, regardless of length of exposure. Though the MAC is 500 p.p.m., exposure to 300 p.p.m. has caused intoxication, even with protection. Death was caused by respiratory arrest and depression of the central nervous system, and possibly ventricular fibrillation; the myocardium can be sensitized by the aromatic hydrocarbons in petrol mixtures so that even endogenous epinephrine can precipitate fibrillation. The patient had exposed himself to this risk as he wanted to leave early for an important engagement. [See GERARDE, p. 1108.] *M. Patricia Fitzsimons*

GHOSH, P. K. **Effect of Sulphuric Acid on Teeth in a Battery Factory.** *Indian J. Indust. Med.* 1961, June, v. 7, No. 2, 59–62, 3 figs. on 2 pls.

This is an account of damage to the teeth of workers in the "forming" department of an accumulator battery factory in the Calcutta area, caused by exposure to sulphuric acid vapour which is discharged into the atmosphere in the process of "forming" the accumulator plates.

The forming department was started in 1946 with a staff of 12 workmen. With increasing workload the number of men had gradually to be increased to 48 in 1960 but the size of the building remained as it was in 1946.

It was not till 1960 that any complaints about their teeth were made by the workers. At a detailed dental examination 12 of the 48 workers made complaints. The earliest dental manifestation was a "tingling sensation with cold water". Later there appears a brownish discoloration and then erosion of the teeth. These changes were seen only on the upper and lower incisors, and were restricted to the labial surface of the teeth and their cutting edges.

It would appear that the reason why no dental complaints were made till 1960 was that about that time the work done had increased greatly, causing the concentration of acid in the atmosphere to become much greater. No chemical determination of this concentration could be made.

The length of exposure to the acid did not seem to influence the degree of damage to the teeth for whereas 3 out of 15 men who had worked for a year or less had dental damage, only 1 of 7 men exposed for 7–10 years showed this dental change.

Preventive measures which are considered to have had a good effect were the introduction of exhaust ventilation and the addition of a petroleum detergent to the forming tanks to lessen the "gassing" on the surface.

[See also this *Bulletin*, 1961, v. 36, 536.]

M. E. Delafield

SALEM, H. & AVIADO, D. M. **Inhalation of Sulfur Dioxide. Comparative Behavior of Bronchiolar and Pulmonary Vascular Smooth Muscles.** *Arch. Environment. Health.* Chicago. 1961, June, v. 2, No. 6, 656–62, 5 figs.

Tracings show the simultaneous responses of pulmonary vessels and bronchioles, and other cardiovascular measurements, in 11 anaesthetized dogs subjected to 2 or more inhalations of SO_2 at concentrations of 200–850 p.p.m. in air at constant pressure, 10–15 mm.Hg, for 1–4 minutes. Absorbed SO_2 averaged 86% (range 78·89–98·98). To assess the role of vagal bronchoconstrictor fibres, 4 of these dogs were intravenously injected with 1 mgm. per kgm. of atropine sulphate; absorbed SO_2 averaged 79% (57·79–96·19). (Exhaled SO_2 was measured.) A table lists amounts of absorbed SO_2, and lung moisture contents—4 dogs above normal—and lesions, the last possibly caused by respiration and perfusion pumps as well as by SO_2.

The SO_2 caused: pulmonary vasoconstriction; bronchoconstriction preceded and followed by dilatation; increased pulmonary artery pressure; depression of myocardial contractile force with bradycardia; shock. The constriction of pulmonary veins supplements the action of bronchiolar muscles and respiratory reflexes in reducing the amount of SO_2 absorbed and carried into the systemic circulation.

The biphasic response in bronchomotor tone was found 15 times in 10 dogs after absorption of 105–810 p.p.m. (1·5–15·5 gm.) SO_2, but in 3 instances, only bronchodilatation. The atropine caused dilatation twice, the biphasic response twice and had no effect on tone in 4 instances. Constriction sometimes persisted, even after atropine; this may have been due to histamine release or to local effect of sulphurous acid on smooth muscle. The immediate dilatation was probably a local response to SO_2. Tone returned when SO_2 was stopped.

The accompanying pulmonary arterial pressure varied: rise, fall or both. In other experiments a rise in pulmonary arterial perfusion pressure (perfusion was with blood from right atrium) demonstrated vasoconstriction, even after atropine; and this was not caused by pulmonary blood flow which was not altered by SO_2. After SO_2 was stopped there was a reduction in flow, even with atropine, related to depression of heart rate and force and of aortic pressure. *M. Patricia Fitzsimons*

LAFLÈCHE, L. R., BOIVIN, C. & LEONARD, C. **Nitrogen Dioxide—A Respiratory Irritant.** *Canadian Med. Ass. J.* 1961, June 24, v. 84, No. 25, 1438–43, 10 figs. [10 refs.]

This paper contains a full report on a case of nitrogen dioxide poisoning which is preceded by an account of some of the literature on this poisoning in workers in industrial and chemical laboratories, in persons exposed to the inhalation of gases given off by the decomposition of roentgen films of the nitrocellulose type and more recently in workers in silos who suffer the condition of "silo-filler's disease" [this *Bulletin*, 1956, v. 31, 917; 1957, v. 32, 43; 1958, v. 33, 677] through the inhalation of the nitrogen dioxide fumes which are given off within a week of the silo being filled.

The case report is that of a 16-year-old boy engaged in farm work who was admitted to hospital because of persistent cough with obvious respiratory distress, some fever, cyanosis and sputum tinged with blood. There was a history of pulmonary tuberculosis 8 years before and hence he was treated with streptomycin, Rimifon, para-aminosalicylic acid and antibiotics although there was no bacteriological evidence of tuberculosis. There is a full clinical account of his grave condition with also a number of X-ray pictures of the chest. Improvement in the clinical state did not start till the 27th day in hospital but by the 41st day the boy had become afebrile, acyanotic and without respiratory distress. The radiological examinations of the chest which had revealed confluent opacities radiating outwards from the hilar regions now showed resolution of the lesions but as micronodular densities were still present it was decided to carry out a lung biopsy for diagnosis. The operation was carried out under anaesthesia induced by intravenous anaesthetics and maintained on nitrous oxide. 4 hours after operation the clinical picture had become comparable to that on admission but the fever, cyanosis and dyspnoea lasted only a few days.

It was only after the lung biopsy was made that exhaustive interrogation of the boy revealed that he had been exposed to "nitrogen dioxide and related fumes" on 4 occasions—when (1) working with a fertilizer containing nitrates, (2) filling a silo, (3) burning as a prank a tin of shoe polish which contains nitrites and nitrobenzene, and (4) he was anaesthetized with nitrous oxide.

This history and the X-ray findings of "interstitial pneumonia with alveolitis and bronchiolitis obliterans" led to the diagnosis of nitrogen dioxide poisoning.

M. E. Delafield

SCHEPERS, G. W. H. **The Pathogenicity of Glass Reinforced Plastics. Experimental Inquiries by Injection or External Application Techniques.** *Arch. Environment. Health.* Chicago. 1961, June v. 2, No. 6, 620–34. [11 refs.]

4 previous studies [see this *Bulletin*, 1959, v. 34, 57, 135, 1022] in which the author has participated have shown that 2 varieties of Fiberglas-reinforced polyester resin plastics behaved generally like comparatively inert substances when inhaled by experimental animals for a prolonged period of time. The present study is concerned with the local tissue effects of the plastics or their components. The animals used were rats, guineapigs and rabbits and the substances investigated were (a) Fiberglas reinforced polyester plastic with a calcium sulphate filler, (b) Fiberglas-reinforced polyester plastic with a calcium carbonate filler, (c) polyester plastic with a calcium carbonate filler but without the Fiberglas, (d) Fiberglas (pulverized and ball-milled) without plastic, (e) polyester plastic without either the filler or the glass reinforcement and (f) flake glass (pulverized and ball-milled). The substances were introduced by intratracheal and intravenous injection, cutaneous infriction, and ocular instillation. Details are given of the materials and methods used and the results are summarized in 12 tables.

Spontaneous deaths among the animals, particularly the rats, were high but it is pointed out that intratrachea

njections frequently result in heavy casualties. Pneuoconiotic lesions were macroscopically more impressive n animals that received the product with the calcium arbonate filler than in those which were given Fiberglas lastic with calcium sulphate filler and this was also oted on histological examination; focal granulomata ormed more readily and persisted longer in the animals o which Fiberglas with calcium carbonate filler was dministered. In other respects the effects of the three arieties of dust differed little, but vesicular emphysema eveloped in a large proportion of the animals, which vas attributed to the bronchitis and bronchiolitis induced y the Fiberglas plastics. However, these lesions appeared vhen only the plastic (plus calcium carbonate filler) was iven so that the Fiberglas component was not the priiary factor in their production. The injection of Fiberglas lone gave rise to few detectable responses and this was lso true when polymerized resin alone (without Fiberglas r filler) was given, but in the latter case most of the dust articles were trapped in the liver and spleen where they ongregated within extremely large multinucleated cells. hese results are due to the ready phagocytosis of plastic, iberglas plastic, and Fiberglas dust, but 50 mgm. of iberglas plastic (with calcium sulphate filler) when mplanted subcutaneously in rats caused lesions which egressed only slowly during a period of 12 months. The lass fibres persisted in the tissues and although the alcium sulphate was readily evacuated, most of the lastic remained during the first 8 months. The milled ake glass did not result in fibrosis on intratracheal injecon and the glass particles rapidly disappeared. Granuoma formation, emphysema, bronchial and bronchiolar amage occurred much less frequently and when injected ntravenously the flake glass also behaved as do inert ubstances. Rubbing flake glass into the skin resulted nly in slight erythema and when dropped on the cornea o corneal or conjunctival reactions were present at the nd of 3 months.

Some of the limitations of the intratracheal technique re discussed but in spite of them the author feels that aluable evidence has been obtained in these experiments, nd it can be said that all these substances are comparavely inert. However, the combination of filler plus lastic (without glass reinforcement) was more active than ither agent separately and the combination of the plastic ith the calcium carbonate potentiates the effect of both ubstances. The fibrosis which occurs as a result of mplantation may be due to the retention of glass fibres nd on the analogy of experience with asbestos fibres nd pulverized asbestos [*ibid*, 1951, v. 26, 511] it is sugested that the elongated character of the Fiberglas ragments may be more influential in producing the local ssue reaction than is the chemical composition of the ubstance. *G. F. Keatinge*

DE MATTEIS, F., PRIOR, B. E. & RIMINGTON, C. **Nervous and Biochemical Disturbances following Hexachlorobenzene Intoxication.** *Nature.* 1961, July 22, v. 191, 363–6, 2 figs. [13 refs.]

This investigation, carried out in University College Medical School, London, arose out of the reports that the outbreaks of cutaneous porphyria which have occurred recently in Turkey are due to the consumption of bread made from wheat dressed with hexachlorobenzene (C_6Cl_6) and intended for sowing [*Trop. Dis. Bull.*, 1961, v. 58, 130, 131]. [Hexachlorobenzene is a fungicide and quite different from the insecticide benzene hexachloride (gamma-BHC, $C_6H_6Cl_6$).]

Rabbits, guineapigs, mice and rats were fed a diet containing 0·5% hexachlorobenzene. Guineapigs and mice were the most sensitive species, developing severe poisoning in 8–10 days. In the rabbit and rat symptoms appeared after about 6 weeks although poisoning, fatal in 3–4 days, was produced in week-old rats allowed to suckle a mother given the diet containing hexachlorobenzene. In all species the poisoning was fatal and the main symptoms were neurological although no morbid anatomical basis was found for them. Rats also showed cutaneous lesions. Porphyrin metabolism was affected in all the species but there was no strict correlation between the degree of this affection and the severity of the nervous symptoms.

The neurological changes were characterized by weakness, fine or coarse tremor leading to convulsions or clonic contractions. These effects occurred rapidly, before porphyrin metabolism was severely affected. The changes in the latter are described in detail and differ slightly between the different species. There was an increase in urinary porphyrin and porphobilinogen excretion with a rise in delta-amino-laevulic acid excretion towards the end of the experiment. The disturbance was most marked in the rabbit where it most closely resembled the condition in man. It is thought that the porphyria is of the hepatic type with possible interference to the uroporphyrinogen decarboxylating mechanism.

H. B. Stoner

BEHEYT, P., LEBRUN, A., CERF, J., DIERICKX, J. & DEGROOTE, V. Étude de la toxicité pour l'homme d'un insecticide organophosphoré. [**Toxicity for Man of an Organophosphorus Insecticide**] *Bull. World Health Organization.* Geneva. 1961, v. 24, Nos. 4/5, 465–73, 4 figs.

[An abstract of this paper appeared in the *Tropical Diseases Bulletin*, 1961, v. 58, 1257.]

HEARN, C. E. D. **Trithion Poisoning.** *Brit. J. Indust. Med.* 1961, July, v. 18, No. 3, 231–3.

The author reports 19 cases of poisoning by the organophosphorus insecticide trithion (O,O-diethyl-S-*p*-chlorophenylthiomethyl phosphorodithioate) among workers on a sugar cane estate in Trinidad. The patients showed the usual range of symptoms and signs produced by this type of compound, none died and the 7 who required treatment were adequately controlled by atropine. None developed any delayed neurological lesions. Whole blood cholinesterase levels were determined by a rapid field method in 9 cases and found to be between 25 and 62·5% of the normal.

535 workers were at risk on the estate; 60 of these worked in the mixing shed but with one exception the

cases occurred among those applying the 2% trithion in limestone dust to the sugar canes. *H. B. Stoner*

STUR, O. & ZWEYMÜLLER, E. Diagnose und Therapie der akuten γ-Hexa-chlor-cyclohexan-Vergiftung bei Kleinkindern. [**Diagnosis and Treatment of Acute Poisoning due to gamma-BHC in Children**] *Deut. med. Woch.* 1961, Aug. 4, v. 86, No. 31, 1474–6, 1495. [16 refs.]

Reports are presented here of cases of accidental poisoning by gamma BHC in 3 children, aged 1½–2 years. All had ingested the chemical in the form of Jacutin, a trade preparation.

Signs of poisoning appeared within an hour of taking the substance and consisted of restlessness passing into lessening or loss of consciousness, tonic and clonic spasms and sometimes cyanosis. On examination 2 of the children were found to have dilated pupils and some lessening of the activity of reflexes. All the children recovered.

The main points in treatment are immediate gastric lavage and administration of saline laxatives. The spasms are best treated by intravenous injection of calcium thiosulphate in a 10% solution which is preferable to giving barbiturates which may increase the degree of collapse seen in severe cases. It is emphasized that giving fats or oils of any kind is strongly contra-indicated because gamma BHC is soluble in fats though sparingly soluble in water.

These 3 cases were mild but gamma BHC poisoning may be severe and cause death or be of chronic type.
M. E. Delafield

ROSIVAL, L. & RAJNOHA, F. Ochrana zdravia pri výrobe intratiónu. [**Health Protection in the Production of Intrathion**] *Československ. Hyg.* Prague. 1961, v. 6, No. 5, 287–94, 1 graph. [15 refs.] English summary.

The authors have studied the public health problems arising in the industrial production of Intrathion, an organophosphorus insecticide containing as active principle O : O-dimethyl-S-(ethylmercaptoethyl)-dithiophosphate. Toxic effects can be detected by serial determinations of the plasma and red cell cholinesterase activity, and persons in whom low values are obtained should be withdrawn from work. Ventilation of the working places should be efficient, and the maximum permissible concentration in the air is 0·0001 mgm. per cu.m.
D. J. Bauer

ČERNÁ, Věra. Enzymatické stanovení reziduí fosfotionu po oxydaci. [**Enzymatic Estimation of Residues of Phosphothion after Oxidation**] *Československ. Hyg.* Prague. 1961, v. 6, No. 5, 295–301, 1 graph. [12 refs.] English summary.

A method has been developed for determining residual concentrations of phosphothion (a Czechoslovakian preparation containing 35% malathion) on the basis of inhibition of cholinesterase activity, with preliminar oxidation in order to increase the inhibitory activity an thereby raise the sensitivity of the method. Phosphothio dissolved in benzene is mixed with the oxidizing re agent (1 part of 30% hydrogen peroxide and 5 parts c glacial acetic acid) and heated in a water-bath at 75°C for 30 minutes. After cooling, benzene is added to re store the initial volume, and water is then added an the mixture is shaken. The layers are allowed t separate and a portion is removed and evaporated t dryness. Water, buffer and cholinesterase (in the forn of horse serum) are then added, and the mixture i incubated at 37°C. for 60 minutes to allow inhibitio of the enzyme to take place. Acetylcholine is the added, and after further incubation for 60 minutes th extent of the enzyme reaction is determined by measur ing the fall of *p*H produced by the acetic acid liberated The degree of inhibition produced by the phosphothio is determined by comparison with a control without in hibition. A standard curve can thus be constructed, th percentage inhibition being linearly related to th amount of phosphothion up to at least 6·6 μgm.

The sensitivity of the method was found to be in creased 10-fold by the oxidation procedure, and it gav satisfactory results in model experiments in which th concentration of phosphothion was determined in plan extracts. The product obtained by the oxidation c phosphothion was investigated by paper chromato graphy. *D. J. Bauer*

LEVEQUE, J., TROTEMANN, S. & DELONS, S. Deux intoxica tions par les esters phosphoriques traitées par u réactivateur des cholinestérases (sulfométhylate de l N méthyl α pyridylaldoxime). [**Two cases o Poisoning by Phosphoric Esters treated by Cholinesterase Reactivator (N Methyl α-Pyridy aldoxime Sulphomethylate)**] *Maroc Méd.* 196 Apr., v. 40, No. 431, 405–12, 1 fig. & 2 charts. [1 refs.]

After an account of the pharmacology of organi phosphates, the mechanism of anticholinesterase actio and the rationale of treatment of poisoning by choline terase reactivators, the compound 7676-RP is describe It is N-methyl α-pyridylaldoxime sulphomethylate, white crystalline powder, more soluble than the corre ponding iodomethylate and it is potentiated b administration of atropine sulphate intravenously. was used in a 2% aqueous solution, with success in patient and with good effect on cholinesterase values i both patients. The paper ends with the warning, s often repeated, that the widespread use of organi phosphorus insecticides is dangerous, if only becaus distributors do not stress the danger and agricultura workers have gained a false sense of security from lon years' use of less toxic pesticides. Regulations nee tightening.

Clinical, biochemical and therapeutic details are s forth in tables and figures at the times they occurre in a woman aged 25, an attempted suicide who re covered, and an agricultural worker, aged 20, who die suddenly from a return of respiratory embarrassment 1 hours after admission in coma. His respiration an

cyanosis had improved during the first hour with 2·5 mgm. atropine and 400 mgm. 7676-RP; the pupils dilated and coma gave way to agitation; 200 mgm. 7676-RP was given in 2 hours; red cell cholinesterase activity had risen from 7% of normal to 55% and serum activity from 1% to 9%. The woman had 4·5 mgm. atropine and 400 mgm. 7676-RP during the first hour, and 1 and 200 mgm. 1 hour after admission when she came out of coma and vomited some purple powder. Coma and respiratory distress were too severe for gastric lavage. Respiration improved during the first hour and pupils began to dilate 2 hours later. Vomiting recurred. The 7676-RP was repeated 4 and 14 hours after admission. Red cell cholinesterase activity rose from 12% of normal to 65% in 2 hours and to 90% in 10 days; serum activity rose from 8% to 19% in 2 hours and slowly increased to 45% in 10 days. Serial results are given for both patients; Michel's electrometric method was used and results are compared with those obtained by other methods and by other forms of treatment. *M. Patricia Fitzsimons*

MÜLLER, J., DAVID, Alois, REJSKOVA, Marie & BREZIKOVA, Dagmar. **Chronic Occupational Exposure to Strontium-90 and Radium-226.** *Lancet.* 1961, July 15, 129–31, 4 figs.

This work arose from the occurrence of several cases of radium dermatitis among luminous-dial painters in the district of Prague.

At the factory in question a new type of paint had been introduced. It contained 1·2 mc of ^{90}Sr per gm. of paint plus a little radium. The foreign producer of the paint had declared it harmless so it came into the country as "paint" and not as radioactive material; hence the preventive measures of the Hygiene Epidemiological Service were not enforced.

On inspection it was found that a number of the personnel were affected; 34 of them were selected for admission to hospital. Measurements were undertaken of their urinary ^{90}Sr excretion and in 10 subjects their body burden of ^{226}Ra.

Those who were taken off work showed a gradual decline in the Sr content. This is shown graphically and is said to be satisfied mathematically by an equation consisting of 3 exponential expressions. The general character of the curve leads one to think that a simpler equation would satisfy the conditions.

Suitable precautionary measures at the factory and elsewhere were introduced among which the following may be noted:—

1. The use of radioactive paints containing ^{90}Sr was declared illegal.

2. To prevent uncontrolled use of radioactive substances radioactive isotopes are only to be distributed centrally.

3. Workshops for assembly and repairs are to be considered part of the controlled area and hygienic measures enforced in such work.

Apart from the finding of ^{90}Sr in the body haematological studies revealed signs of irritation of erythropoiesis, such as reticulocytosis and hyperplasia of the erythroblast series in the bone-marrow. *S. Russ*

GUSEV, N. G. **[Maximally Permissible Ionizing Radiation Levels accepted in the USSR in 1960]** *Gigiena Truda i Professional'nye Zabolevaniya.* Moscow. 1961, v. 5, No. 4, 8–14. [11 refs.] [In Russian.] English summary (6 lines).

A new code of rules for work with radioactive substances and sources of ionizing radiation was introduced in the USSR in 1960. A part of this was "PDU–1960" (Maximum Permissible Levels—MPL). The paper presents a short account of "PDU–1960" and the sources used in its compilation, when it differs from the recommendations of the International Commission on Radiological Protection, 1959 (ICRP).

1. *Maximum Permissible Doses.* The Soviet regulations do not allow increased doses for inhabitants near to controlled areas [ICRP, para. 36 B (c)].

2. *External Levels of Ionizing Radiations.* ICRP is stated to be deficient with regard to such radiations of high energy. MPL are available for thermal and intermediate neutrons, fast neutrons, very fast and super fast neutrons; also for protons, deutrons, tritrons and multiple charged ions. Details are not given but there are references to the sources used for calculations.

3. *Change and Additions in "PDU–1960" compared with ICRP–1959 for MPL of Radioactive substances in water and air.*

"PDU–1960" does not contain MPL in water for a 40-hour week occupational exposure but controls the content of isotopes in reservoirs and water supply. This is based on the Maximum Permissible Dose (MPD) for the general population.

ICRP contains for every isotope 5 to 10 values of MPL for water and air depending on the method of calculation applied to different critical organs. "PDU–1960" gives only the minimum value.

"PDU–1960" takes into account general toxic properties as well as radioactive effect in calculating MPL, which may be important for isotopes of long half life [See ICRP Report of Committee II, Section III (1) (j)].

Where the spleen is the critical organ, the dose has been calculated as for the gonads and crystalline lenses group of organs.

The ICRP list of radionuclides has been supplemented by 20 isotopes, *e.g.* ^{11}C, ^{15}O, ^{12}N, etc., etc.

4. *Discussion of Results.* There is criticism of the method of calculation of bone-seeking isotopes, *e.g.* ^{89}Sr, ^{90}Sr, etc., which are based on the maximum loading for uranium 0·1 μc derived from the U.S.A. luminizing tragedy. The author makes the following observations.

(1) In the U.S.A. the spontaneous frequency of occurrence of bone tumours from 0·1 μc of radium would require statistical treatment of data from hundreds or thousands of persons. Further in latter years the average duration of life has increased.

(2) 99% of radium in the body is deposited in the skeleton. But other bone seekers can irradiate other organs. For example, ^{95}Zr is also found in the spleen and this organ might receive an excessive dose (using the criteria noted in 3 above).

(3) Radium is essentially an emitter of alpha particles, the particles having a track of about 40 microns in bone. Whereas the track of beta particles, *e.g.* from ^{90}Sr

is 160 microns. It is concluded that bone marrow (a more sensitive tissue) may thus receive an enhanced dose.

(4) Thus in the ICRP the permissible load for ^{90}Sr is 2μc, corresponding to 0·1μc of radium. The work of Finkel (1956) is quoted, showing that in mice with 0·1μc of radium in the bones death from cancerous tumours was 10%, but with a concentration of 2μc of ^{90}Sr it was 45%.

(5) According to ICRP the difference of MPD for bone as a critical organ compared with bone marrow should be a factor of 6. But calculations are cited showing that the difference in dose from ^{90}Sr lies between 1·3 and 2·2.

(6) The relationship of stable strontium to calcium in the bones of different persons has a "lognormal" character.

(7) The author refers to an English report (presumably "The Hazards to Man of Nuclear and Allied Radiations" 1956 HMSO Lond. Cmd. 9780 para. 81, this *Bulletin*, 1956, v. 31, 1054) that precancerous cystic bone changes develop with a concentration of radium of 0·4–0·5 μc/body. (In the reference just quoted this refers to "early non-cancerous cystic changes in the bone".) The article continues—"It is difficult to agree that the difference between the maximum permissible level of radium 0·1μc and that pointed out above should be only by a factor of 5".

The author points out the lack of knowledge of the "strontium cycle" in nature, but that it is known that insignificant concentrations of these isotopes in the air can, as a result of the natural cycles, lead to a high degree of accumulation in the human body.

W. R. Lee

KURLYANDSKAYA, E. B. [**Some Questions pertaining to Biological Substantiation of Maximally Permissible Concentrations of Radioactive Substances**] *Gigiena Truda i Professional'nye Zabolevaniya.* Moscow. 1961, v. 5, No. 4, 14–18. [In Russian.] English summary (8 lines).

The history of the establishment of maximum permissible levels of radiation is briefly reviewed. The new rules for the USSR, following on the latest recommendations of the ICRP include 0·1 r per week and 5 r per year.

Biological substantiation of MPC is needed. The starting point for establishment of MPD is that it does not cause either acute or chronic disease. This concept is contrasted with the statement "the permissible dose is that which in the light of present knowledge carries a negligible probability of severe somatic or genetic injuries" [ICRP (1959) para. 30. The word "severe" is put into italics by the author]. Next para. 31 of ICRP, 1959, is quoted "for example leukaemia that might result from exposure of individuals to the permissible dose would be limited to an exceedingly small fraction of the exposed group" [again, the author puts "permissible dose" in italics].

Results are quoted of experiments to substantiate the calculated maximum permissible concentrations. Over a period of 2 years ^{60}Co was introduced into rabbits [it is not stated in what form or by what route] in an amount 100 times in excess of the MPD: it was found that the dose of general radiation to the body exceeded the MPD by 5 times and in the critical organ, the liver [*sic*], by 14 times. When the amount of ^{60}Co was increased by a factor of 10 so "exceeding the MPC by 1000" the total body dose exceeded the maximum permissible by 25 and the liver by 80 times. Despite this experiments have proved that an increase of MPL in the body by 5–10 times leads to pathological changes (this has been shown for ^{89}Sr, ^{134}Cs, ^{106}Ru, ^{60}Co, ^{59}Fe etc.).

Experiments on insoluble compounds, given by the intratracheal route into rats, have given rise to the impression that there is no threshold for initiation of lung tumours; thus ^{59}Fe as insoluble oxide produces cancer at a dose of 486 rad which is not produced by other isotopes (^{32}P, ^{198}Au). It is suggested that the chemical and biological properties of the isotope may be important. Another point made with regard to soluble and insoluble compounds is that certain isotopes, introduced into the lungs in soluble form, form only partly soluble complexes with proteins and this can lead to retention in the tissue—9% of ^{59}Fe citrate is found in the lungs 30 days after intratracheal introduction.

[This paper is essentially a plea for biological verification by long continued experiment of the Maximum Permissible Concentrations which have been reached by mathematical methods.] *W. R. Lee*

GORODINSKY, S. M, PANFILOVA, Z. E. & PROMPTOV, N. A. [**Protective Garments of Synthetic Fibre for dealing with Radioactive Substances**] *Gigien Truda i Professional'nye Zabolevaniya.* Moscow 1961, v. 5, No. 4, 32–9, 5 figs. [In Russian.] English summary (8 lines).

Protective garments made of cotton are not easily cleansed from contamination with radioactive substances and the fabric will not withstand strong solutions which may be used for washing. The incorporation of an airtight film on to the fabric makes for hot and uncomfortable working.

The properties required of materials for these protective garments are cited as: (1) minimal absorption of radioactive substances, (2) practically complete cleansing from radioactive contamination by washing, (3) mechanical strength, (4) stability against strong solutions used for decontamination, (5) permeability to ensure adequate thermoregulation, (6) absence of unpleasant smell or skin irritation.

Among a number of synthetic fibres tested was Lavsan (described as polyethylene terephthalate). Tables show its thermal stability, high durability, low hygroscopic qualities and resistance to mineral acids (including 50% H_2SO_4, 35% HCl and 45% HNO_3, it was dissolved by 94% H_2SO_4 at 20°C. in 20 hours).

A number of materials of differing type, number of threads, type of weave, etc. were made and tested for absorption and ease of removal of radioactive substances (including ^{90}Sr, ^{60}Co, ^{131}Cs). Pairs of cloth samples were contaminated, one was cleaned with an "oxidizing-reducing" solution and the other with a

acid solution. [Fuller details of method and composition of solutions are given.] Both methods are reported as equally efficient—the more easily used acid method (employing HCl) was used in subsequent work.

It was found that Lavsan showed the lowest percentage of residual activity (0·6%). Garments manufactured from continuous filament accumulated residual radioactivity less than those made from staple fibres. Differing character of warp, weft and weave did not materially affect these properties of continuous filament Lavsan fabrics. Dyes and pigments introduced into the material during manufacture did not alter these properties of absorption and ease of removal.

Two types of Lavsan fabric were compared with cotton with respect to strength, weight, permeability, etc. Lavsan was 2–3 times as durable as cotton. The continuous filament Lavsan fabric had a permeability of 202 litres/m²/second.

A number of overalls and suits were made of Lavsan fabric and worn by 4 different subjects, carrying out work of average physical effort for 2 hours at 45°C. Physiological measurements included skin temperature, sweat production, heart rate, body temperature and gaseous exchange. There was little difference in the results when cotton or Lavsan garments were worn (the sweat secretion was 850–900 gm.).

An 18-month trial of suits of this material under actual working conditions showed that they wore longer than cotton and required less washing solution.

[Lavsan is a Polyester, by-product of coke and petroleum.] *W. R. Lee*

BEZZUBOV, A. D. & KHATINA, A. I. **[Pectin as a Prophylactic Agent in Strontium Intoxication]** *Gigiena Truda i Professional'nye Zabolevaniya.* Moscow. 1961, v. 5, No. 4, 39–42. [In Russian.] English summary (7 lines).

Different compounds have been tried for use for the elimination of radiostrontium from the body. They have not been effective or without danger. It was decided to investigate a foodstuff which could be given as a "curative-prophylactic diet".

Pectin, found in a number of vegetable sources, has a polygalactic chain and "anhydrogalactic acid". The pectic acids form salts, which are precipitated, with the alkaline earth metals.

A method is described of reacting pectin with strontium at different *p*H values and the quantities of strontium in the filtrate and precipitate were estimated. At *p*H 3·4–3·6 about one-half the strontium was taken up in the precipitate. The experiment was repeated at the *p*H of gastric juice (about *p*H 2) and only about one-quarter or one-fifth was taken up in the precipitate. For intestinal juice (*p*H about 7·6) some four-fifths of the pectin is taken into the precipitate.

Based on these findings, recommendations were made for biological testing. It is stated that these proposals have been confirmed. *W. R. Lee*

See also p. 1090, TAKAMURA & UEDA, **Hematologic Findings in Children exposed to A-Bomb Radiation in Utero in Hiroshima.**

RUBANOVSKAYA, A. A. **[The Influence of Pectin on the Radiostrontium Absorption from the Gastrointestinal Tract under Experimental Conditions]** *Gigiena Truda i Professional'nye Zabolevaniya.* Moscow. 1961, v. 5, No. 4, 43–7. [In Russian.]

The English summary appended to the paper is as follows:—

"To perform the studies experiments on rats were staged. In one series of tests pectin was given to rats per os (1·5 ml 2% solution) 1–2 minutes or 1 hour prior to the introduction of Sr^{90} into the stomach (10–12 mcc). The determination of Sr^{90} level in bones (femur) and in excreta of rats showed that pectin administration effected 1–2 minutes before that of Sr^{90} sharply reduces the isotope deposition in the skeleton and enhances its excretion with feces. When administered 1 hour before Sr^{90} pectin proves somewhat less effective.

"In another experimental series pectin was being added to a standard diet of young rats, which received daily, for a period of 8 days, 2 mcc Sr^{90} per os. A pectin enriched diet tended to reduce the Sr^{90} deposition in the skeleton of rats by 56%.

"To explain the mechanism underlying the action of pectin the effect of pectin solution, broken down with pectinase enzyme partially to the level of galacturonic acid, was studied under similar conditions. It was found that, with respect to radiostrontium, the broken down pectin proved ineffective. It is, apparently, only the integral pectin molecule that is able to bind radiostrontium."

Food and Nutrition

The order in this section is:—**General; Foodstuffs; Food Poisoning.**

GENERAL

SEVERS, D., WILLIAMS, T. & DAVIES, J. W. **Infantile Scurvy—a Public Health Problem.** *Canadian J. Pub. Health.* 1961, May, v. 52, No. 5, 214–20. [13 refs.]

In response to a circular enquiry, 35 doctors in the Province of Newfoundland reported a total of 39 cases of infantile scurvy, treated at home and in cottage hospitals in 1959, and 38 others were admitted to the larger hospitals. The 38 admissions to the larger hospitals cost a total of $13,550 over 710 patient-days. It is estimated that the total of 77 cases represents an incidence of 250 per 100,000, among the approximate population of 30,000 children aged 0–2 years; or about twice the

incidence of paralytic poliomyelitis in the same age-group during 1959, when poliomyelitis was epidemic. There is also evidence that infantile scurvy is increasing in other parts of Canada, and this contrasts with a report of only 16 cases in Great Britain during the past decade.

The Department of Health makes a free issue through cottage hospitals and baby clinics of concentrated orange juice, to mothers with infants under 1 year of age. Public demand has been met by the provision of 600 gallons per year at a cost of $6,000; this is calculated to provide 30 mgm. of ascorbic acid per day to only 500 infants. Investigations demonstrated that storage at warehouse-temperatures, and at room-temperature in the home could account for a 65% reduction in ascorbic-acid content of the juice. It is concluded that in these conditions orange juice is an unreliable and uneconomical prophylactic against infantile scurvy.

Some information was obtained relating to 40 of the 77 cases, indicating why the children had not received ascorbic acid in their diet. Of a number of factors, the most important were maternal ignorance, and that no children were breast-fed, all being fed on evaporated milk. Lack of medical care was not a factor, for 70% had received Salk vaccine, and 80% were born in hospitals.

Infantile scurvy commonly developed between 6 and 12 months. This suggests that the tissues at birth contain sufficient ascorbic acid to meet metabolic needs for at least 6 months. In breast-fed children supplies are replenished and not depleted. Before Confederation with Canada, and during the depression, breast feeding in Newfoundland was more common, so was the use of fresh cows' and goats' milk and home-grown foods such as potatoes, cabbage and turnips. More ready availability of cash has brought increased dependence on manufactured foods and evaporated milk. In these conditions the fortification of evaporated milk with vitamin C would be an effective public health measure which should replace the issue of free orange juice. This could be expected to produce immediate and permanent benefit to all at risk. The more intensive education of parents in proper methods of infant feeding needed can only succeed when advice given by public health officials and private physicians is co-ordinated and consistent. *T. P. Eddy*

BROCK, J. F. **Recent Advances in Human Nutrition with special reference to Clinical Medicine.**

This book was reviewed on p. 510.

FOODSTUFFS

Composition and Bacteriological Control

BARNITZKI, Ruth. K průkazu bakteriálních toxinů v mase barevnou reakcí. [**The Detection of Bacterial Toxins in Meat by a Colour Reaction**] *Československ. Hyg.* Prague. 1961, v. 6, No. 5, 321–4. English summary.

The author has investigated the use of the Kolobolotski colour reaction [KOLOBOLOTSKI, *Veterinarija*, 1956, v. 8, 36] in the detection of bacterial toxins in meat. The method is based upon the reduction in the redox potential of the meat which is caused by the presence of bacterial polysaccharides, and which can be detected by means of a redox indicator. 10 gm. of meat freed from fat is ground with 10 cc. of saline and 10 drops of 0·1 *N* sodium hydroxide solution, and then heated in a boiling water bath to coagulate protein, which is discarded. After cooling, the material is neutralized with 5% sulphuric acid and filtered; to 2 cc. of the filtrate are successively added 3 drops of 0·5% alcoholic brilliant cresyl violet solution, 3 drops of 0·5% silver nitrate solution and 2 drops of 16·8% hydrochloric acid. The mixture is well shaken and 1% potassium permanganate solution is added in drops. If the liquid is decolourized or becomes pink after 3 drops the result is negative, whereas the retention of the original colour of the indicator or the appearance of a greenish-blue colour indicates the presence of bacterial polysaccharide.

The test gave positive results with meat artificially infected with 48-hour broth cultures of *Salmonella typhi-murium*, *Salm. enteritidis*, Salm. Tennessee, *Salm. abortus bovi*, Salm. Newport, *Salm. anatum*, Salm. Dublin, *E. coli*, (including pathogenic serotypes), *Proteus mirabilis*, and *Clostridium botulinum* A, B and D. The test gave negative results with *Staphylococcus aureus*, *S. citreus*, *S. albus*, *Bacillus mesentericus*, *B. subtilis*, and *Chromobacterium prodigiosum*. *D. J. Bauer*

POLAK, M. F. Kippeëiprodukten in Bakkerijen als mogelijke bron van salmonellose bij de mens. [**Hens' Egg Products in Bakeries as a Possible Source of Human Salmonellosis**] *Nederl. Tijdschr. v. Geneesk.* 1960, Dec. 31, v. 104, No. 53, 2660–63. [11 refs.] English summary (7 lines).

During the months from June to September, 1959, 107 samples of hens' egg products were obtained from the bakeries of Utrecht and The Hague, Holland.

In a total of 61 specimens of liquid albumin, 2 were contaminated with *Salmonella typhi-murium* and 2 with Salm. Bareilly. Four samples of albumin crystals of 21 were contaminated each with Salm. Muenchen, Salm. Thompson, Salm. Oranienburg and Salm. Bareilly, and Salm. Bareilly. All other raw materials were free from organisms.

During manufacture the dough was heated to a temperature of 130–230°C. for 11–45 minutes. Although all the mixtures were contaminated with some strain of *Salmonella* before baking, no evidence of this organism

was found after the heating process. The raw products were contaminated in the bakeries. Bacterial counts of *Salm. typhi-murium* found in albumin foam was of an order which would not produce symptoms in healthy young adults but would affect children and old people.

Anne Tothill

PICKUP, J. W. & COLENSO, R. **Paratyphoid B and the Sale of Salmonellae-Infected Desiccated Coconut. A Report on the Administrative Action taken by a County Health Department.** *Med. Officer.* 1961, June 2, v. 105, No. 22, 321–2.

In this paper there is a good account of the action taken by the County Health Department of Worcestershire to cope with the danger to health of imported desiccated coconut infected by bacteria of the Salmonella group. The first information of this potential danger was the receipt by the county of a letter from a large biscuit company in which there was reference to the precautions which had been taken by that company in dealing with the coconut. It was then discovered on enquiry from the Ministry of Health that "sometime previously it had advised the Baking Industry Research Organization that some cargoes of desiccated coconut had been found to contain salmonella infections, including paratyphoid B".

The import of infected coconut continued and it has been reported that for the period July 1959 to March 1960 9% of samples contained bacteria of the Salmonella group.

In Worcestershire in January 1961 3 cases of paratyphoid B infection, Beccles variety, phage-type 4, were traced to ingestion of desiccated coconut in a marshmallow biscuit. Some 300 samples of desiccated coconut sold loose or on biscuits and confections were then taken. 171 of these samples have now been examined and 7 have been found to be infected.

There is a good account of the action taken by the county and due credit is given to the manufacturers of products containing raw coconut for their willing and effective cooperation in withdrawing these products.

The major part, if not all, of this infected coconut comes from Ceylon where, it would appear from the description, the conditions of processing and handling "leave much to be desired".

In the conclusions there are 2 suggestions—(1) "that it would be proper for the Ministry of Health to take control of the immediate issue of information to all local authorities where infected food has a wide distribution" and (2) "that coconut and Ceylonese coconut in particular be permitted entry only under licence and that it must be properly sterilized at our ports before distribution".

M. E. Delafield

PUB. HEALTH LAB. SERVICE. ***Salmonella* Organisms in Animal Feeding Stuffs. Report of a Working Party** [WALKER, J. H. C., Chairman]. *Monthly Bull. Ministry of Health & Pub. Health Lab. Service (directed by Med. Res. Council).* 1961, Apr., v. 20, 73–85.

This is the second report of the PHLS Working Party on *Salmonella* organisms in animals feeding stuffs. The previous investigations of animal feeding stuffs by this working party were reported in 1959 [see this *Bulletin*, 1959, v. 34, 670] in which it was shown that the salmonella contamination rate was considerable in organic materials, obtained from a variety of sources, and used as raw ingredients in preparing complete animal foods.

This second report demonstrates that important changes in the manufacturing procedures have already resulted in significant reductions in the salmonella-isolation rates of many of these products. There does not appear to be any attempt on the part of manufacturers generally to standardize methods of heat treatment of the animal protein ingredients of finished foods and there is still a wide variation in the nature and source of the raw ingredients. In the case of one large manufacturer, the pelleted foods were almost free from salmonellae, and although samples from many different manufacturers showed higher degrees of salmonella contamination the overall figures for pelleted foods were considerably lower than in "meals". The high incidence of salmonellae in certain protein concentrate meals and meat-and-bone meals used as raw ingredients was very significantly reduced when the firms, making these products, introduced various forms of heat treatment.

It is pointed out in the report that many of the salmonella serotypes commonly associated with clinical salmonellosis in man and animals were either not found in, or seldom isolated from, the samples of animal feeding stuffs examined in the present or the previous series of investigations, though there is evidence that outbreaks in man and animals caused by some of the less common serotypes can be traced to contamination of some ingredients of a finished animal food. The epidemiological significance of these investigations is not yet clear and further work is obviously indicated.

H. J. Benstead

ALSTRÖM, I & KÄMPE, Å. Sammanställning av glassundersökningar vid Stockholms stads hälsovårdsnämnd 1957–1959. **[Summary of Investigations of Ice Cream Carried Out by the Stockholm Public Health Authority in 1957–1959]** *Nordisk Hyg. Tidskr.* 1961, v. 42, Nos. 3/4, 81–99. English summary (8 lines).

The results of control investigations of ice cream carried out in Stockholm in 1957–59 are reported and discussed. The tests applied consist of determination of the number of aerobic organisms, coliforms and haemolytic bacteria, and special investigations for the presence of *Salmonella*. During the period under review *Salmonella* was not found, and haemolytic organisms (*Bacillus cereus*) were found only infrequently. Special attention was paid to the examination of soft ice cream, which can be sold in Sweden without preliminary pasteurization. The condition of this product was often unsatisfactory, but has shown a tendency to improvement during the period of application of bacteriological control; thus, whereas 16·7% of 42 samples examined in 1957 were rejected as unfit for consumption, this proportion had fallen to 5·7% of 585 samples in 1959. A similar fall in the percentages of products unfit for consumption was also noted with other types of ice cream.

D. J. Bauer

LOSTIA, G. B. & TROMPEO, G. Controlli e richerche sulla produzione delle bibite analcooliche. **[Controls and Investigations on the Production of Non-alcoholic Drinks]** *Igiene Moderna.* 1960, Nov.–Dec., v. 53, Nos. 11/12, 653–63. [13 refs.] English summary (6 lines).

In Italy, as elsewhere, an ever increasing variety of soft drinks, aerated or otherwise, is being produced and marketed, and regulations were made in 1958 laying down standards and hygiene requirements, including pasteurization. Pending the instalment of heat-treatment plant, the use of benzoic acid and of certain derivatives is temporarily permitted.

The authors here report on their findings after the chemical and bacteriological examination of samples taken at the 8 main factories of Turin. They found a considerable reduction, or even disappearance of the bacterial content and they attribute this to the use of preservative and at the same time to a high concentration of citric acid which inhibits bacterial growth. This acid also promotes the development of moulds which tend to impair the organoleptic properties of the product.

The authors are inclined to favour the use of the preservatives. *J. Cauchi*

MINISTRY OF AGRICULTURE, FISHERIES & FOOD. **The Mites of Stored Food.**

This book was reviewed on p. 959.

See also p. 1196, KELLER *et al.*, **Detecting Viruses on Nonporous Surfaces by Use of the Cotton Swab Technique.**

FOOD POISONING

ALDER, A. E. Erkennung und Behandlung der Pilzvergiftungen. **[Diagnosis and Treatment of Mushroom Poisoning]** *Deut. med. Woch.* 1961, June 9, v. 86, No. 23, 1121–27, 1163. [20 refs.]

In this interesting and informative paper the author comments that the frequency of mushroom poisoning is due to ignorance or carelessness. It is also remarkable that there should be so many cases of poisoning in view of the fact that only about 5 of the numerous kinds of mushrooms are dangerous to life.

In Switzerland during 1919–1958 known mushroom poisoning occurred in 1,980 persons, of whom 96 died. This experience is analysed by number of cases and the scientific names of the mushrooms causing the poisoning and classified in 4 groups:—

1. Slight poisoning—806 cases and no deaths
2. Neurotropic mushroom poisoning—(a) mushrooms containing muscarine—38 cases with 3 deaths, and (b) mushrooms causing symptoms and signs like atropine poisoning—166 cases with 4 deaths
3. Mushroom poisoning dangerous to life—299 cases with 88 deaths
4. Poisoning by spoilt and potentially harmless mushrooms—671 cases with 1 death.

The signs, symptoms and pathology of poisoning in these groups are described and a number of case reports are presented in illustration of these groups, with details of the treatment which should be instituted by the doctor after having ascertained from the clinical picture the group to which the poisoning belongs; it is not necessary for him to know exactly the particular mushroom which has been consumed. *M. E. Delafield*

DOEGLAS, H. M. G., HERMANS, E. H. & HUISMAN, J. **The Margarine Disease.** *Arch. Dermat.* 1961, May, v. 83, No. 5, 837–43, 3 figs. [30 refs.]

During August and September 1960, in the Netherlands, an epidemic occurred of a febrile illness with dermatological manifestations. The outbreak coincided with the introduction of new Planta margarine containing an emulsifier (ME 18), and declined when the product was withdrawn from the market. The illness resembled an epidemic in West Germany in 1958 (*Bläschenkrankheit*) [this *Bulletin*, 1961, v. 36, 577].

50,000 people were affected, of whom 75% were women. [For clinical signs and symptoms and pathological findings see this *Bulletin*, 1961, v. 34, 544, *bis*, 545.]

The author compares the Netherland epidemic with the outbreak which occurred in Germany and finds them essentially similar, although the absence of recurrences in Germany despite the continued use of the product, which was not the case in Holland, has not been explained. *Anne Tothill*

Mycoses and Mycology

The order of diseases and fungi in this section is:—**General; Dermatophytes; Blastomyces; Histoplasma, Sporotrichum, Phialophora; Candida, Cryptococcus; Actinomyces, Nocardia; Aspergillus; Others.**

Papers on Blastomycosis (South American type), Coccidioidomycosis, Mycetoma and Rhinosporidiosis are abstracted in the TROPICAL DISEASES BULLETIN.

SMITH, D. E. & MATTHEWS, J. H. **Treatment of Pulmonary Mycotic Infections with Amphotericin B.** *New England J. of Med.* 1960, Oct. 20, v. 263, No. 16, 782–4.

This article reports the therapeutic results obtained from administering amphotericin B intravenously to 5 patients suffering from blastomycosis, 4 from histoplasmosis and 3 from coccidioidomycosis; and all exhibiting the chronic cavitary pulmonary form of their respective disease. Apparent cure was achieved in the 5 patients with blastomycosis, in 3 with histoplasmosis and in 3 with coccidioidomycosis.

This account, which confirms previous reports of the efficacy of amphotericin B in the treatment of systemic mycoses, includes a table showing results of laboratory studies in relation to different dosages of this drug and also a table setting out the main pathological manifestations, treatment and therapeutic results achieved in regard to the 12 patients whoses cases are reported in this article.

C. J. La Touche

CROTTY, R. Q. **Treatment of Superficial Fungous Infections with Griseofulvin.** *Indust. Med. & Surgery.* Miami. 1961, Aug., v. 30, No. 8, 331–3. [12 refs.]

LOEWENTHAL, K. **The Antifungal Effect of 70% Ethyl Alcohol. A Laboratory Study.** *Arch. Dermat.* 1961, May, v. 83, No. 5, 803–5.

It is commonly thought that 70% ethanol is a good preparation for "cleaning" hair, skin and nails prior to taking specimens for mycological examination, the assumption being that such alcohol is highly bactericidal but only weakly fungicidal, in spite of the fact that there is some evidence to the contrary. The author took fluorescent hairs from scalps naturally infected with *Microsporum audouini* and immersed a proportion of them in 70% ethanol for periods varying from 1 minute to 3 hours.

The hairs were then rinsed twice in sterile saline, dried for 15 minutes at 40°C. and planted on Sabouraud dextrose agar. Similar fluorescent hairs, untouched by alcohol but rinsed, dried and plated in the same way, served as controls. The final count of fungus colonies was taken after 21 or more days

At the final count all the control hairs had given rise to colonies of *M. audouini;* the proportion of alcohol-treated hairs producing fungus depended on the length of immersion. After being in the alcohol for 1 or more hours, no hair gave rise to colonies; at 30 minutes only 3% of the hairs contained viable fungus; at 15 minutes, 27%; at 5 minutes, 50%; at 1–2 minutes, 61%.

It is clear that 70% ethanol is markedly inimical to the elements of *M. audouini* present on hair but whether this is equally true of dermatophyte infections of nail or skin is not established. [Particularly as there is a considerable difference between total and relatively prolonged immersion in alcohol and swabbing with a piece of gauze. In addition, hair infection by *M. audouini* is largely ectothrix in character while nail infections by dermatophytes are noted for their extreme resistance to all forms of topical therapy.] *Ian G. Murray*

NUSINOV, A. E. & IVANOVA, T. S. **[The Effectiveness of Disinfection of Barbers' Lathering Brushes in respect to Pathogens of Dermatomycosis]** *Vestnik Dermat. i Venerol.* Moscow. 1961, v. 35, No. 6, 62–5. [18 refs.] [In Russian.]

The English summary appended to the paper is as follows:—

"In Moscow the disinfection of barbers' lathering brushes is centralized and is effected in two special factories-laundries. Laboratory check-up carried out by the authors disclosed that the spores of *Microsporum lanosum and Trichophyton gypseum* become destroyed upon heating in water up to 75°C. In the above-mentioned factories-laundries the practice of disinfection at a temperature of 90°C. and exposure for 30 minutes completely destroys the pathogenic fungi."

LONDERO, A. T. & RAMOS, C. D. Ocorrência dos dermatófitos "geofílcos" no solo do Rio Grande do Sul (Brasil). **[Occurrence of "Geophilic" Dermatophytes in the Soil of Rio Grande do Sul, Brazil]** *Rev. Inst. Med. Trop São Paulo.* 1961, Mar.–Apr., v. 3, No. 2, 75–7, 4 figs. [12 refs.]

The English summary appended to the paper is as follows:—

"The authors report isolation of *Microsporum gypseum* and of *Keratinomyces ajelloi* from the soil of shady places in parks, gardens and patios from the rural zone of Santa Maria, State of Rio Grande do Sul, Brazil."

LONDERO, A. T., FISCHMAN, O. & RAMOS, C. D. Importância do gato na transmissão do *Microsporum canis,* no Rio Grande do Sul (Brasil). **[Importance of the Cat in the Transmission of *Microsporum canis* in the State of Rio Grande do Sul (Brazil)]** *Rev. Inst. Med. Trop. São Paulo.* 1961, Mar.–Apr., v. 3, No. 2, 81–4. [16 refs.] English summary.

WALKER, Jacqueline & SPOONER, E. T. C. **Natural Infection of the African Baboon *Papio papio* with the Large-cell Form of *Histoplasma*.** *J. Path. & Bact.* 1960, Oct., v. 80, No. 2, 436–8, 7 figs. on 2 pls.

A baboon, *Papio papio*, which had been resident for about 3 years at Winches experimental farm, St. Albans, where it was being kept for helminthological studies, developed lesions due to *Histoplasma duboisii* (large-cell variety of *Histoplasma*). Although these lesions were widespread in the dermal tissues the viscera were not involved. Only one lymph gland contained a cell which was invaded by the organism. The lesions were situated on the right and left buttock, the tail, and on the right and left hand. One of the lesions on the tail exhibited an abscess which involved the bone. Pus aspirated from this contained oval yeast-phase cells 6–12 μ in length and sections of necropsy material from the lesions included cells which were packed with *Histoplasma*.

Cultures were well developed, producing at 26°C., on glucose agar and malt agar, white, cottony colonies. In this respect the isolate differed from that obtained by MARIAT and SEGRETAIN from *Cynocephalus babuin* (*Ann. Inst. Pasteur*, 1956, v. 91, 874) but these authors were likewise unable to find any lesions in the viscera.

C. J. La Touche

BAUM, G. L., GREEN, R. A. & SCHWARZ, J. **Enlarging Pulmonary Histoplasmoma.** *Amer. Rev. Respiratory Dis.* 1960, Nov., No. 5, 721–26, 4 figs. [18 refs.]

Resection of the lung tissue containing "coin lesions" attributed to *Histoplasma capsulatum* was carried out successfully in the case of 4 patients exhibiting "enlarging histoplasmomas". These patients were white males whose ages ranged from 28 to 43 years. No symptoms or physical signs were exhibited by 3 of them, but 1 of them gave a 6 months' history of epigastric pain, chest colds and loss of weight. All gave a positive histoplasmin skin reaction. The histoplasmomas contained *H. capsulatum* in an inactive state.

As a result of these lung resections all the patients recovered and were still in good health at 6 months to 31 months after operation. *C. J. La Touche*

MARIAT, F. & SATRE, J. Action de la sulfaméthoxypyridazine sur quelques actinomycètes aérobies pathogènes. [**The Action of Sulphamethoxypyridazine on Some Pathogenic Aerobic Actinomycetes**] *Bull. Soc. Path. Exot.* 1961, Jan.–Feb., v. 54, No. 1, 63–70, 2 figs. [13 refs.]

Experience in Mexico has shown that infections caused by *Nocardia asteroides* and *Nocardia brasiliensis* may respond to sulphadiazine and diamidinodiphenylsulphone (Dapsone) respectively. These observations led the authors to experiment with the effect of a long-acting sulphonamide on *Nocardia* and *Streptomyces* spp. *in vitro*.

The sulphonamide chosen was sulphamethoxypyridazine and this was incorporated in various concentrations in a solid medium chosen for its lack of antagonism to sulphonamides. The organisms tested were 4 strains each of *N. asteroides, N. Braziliensis, Streptomyces madurae* and *Streptomyces pelletieri* and 2 of *Streptomyces somaliensis*. The results are as follows:—

N. asteroides: apart from a single strain, which bore a close resemblance to *N. brasiliensis*, there was no inhibition at sulphonamide concentrations less than 256 μgm./ml.

N. brasiliensis and Strep. madurae: totally inhibited by sulphamethoxypyridazine concentrations of the order 4–16 μgm./ml. during the first fortnight of incubation. One strain of *Strep. madurae* required 32 μgm./ml.

Strep. pelletieri: totally inhibited at concentrations ranging from 8 to 16 μgm./ml. during the first 10 days of incubation.

Strep. somaliensis: one strain was totally inhibited at 64 μgm./ml. while the other called for 256 μgm./ml.

In all cases partial inhibition occurred at lower concentrations of the sulphonamide.

These findings clearly show that sulphamethoxypyridazine deserves thorough clinical trial in all cases of mycetoma due to these organisms, preferably after mycological identification and drug sensitivity tests.

Ian G. Murray

VORREITH, M., BARES, L., BENES, V. & VANCURÍK, J. Kandidóza centrálního nervového systému rozpoznaná bioptickým vyšetřením. [**Candidiasis of the Central Nervous System diagnosed by Biopsy**] *Časopis Lékařů Českých*. Prague. 1961, Aug. 4, v. 100, No. 31, 966–71, 8 figs. [16 refs.]

The English summary appended to the paper is as follows:—

"In a 17-year-old female patient with symptoms of a developing expansive intracranial process for four months, a granulomatous Candida encephalitis was revealed by biopsy. The patient had suffered since childhood occasionally from thrush and onychomycosis. The diagnosis was confirmed by cultivation tests from the excised cerebral tissue. Though the isolated strain of Candida albicans in vitro was sensitive to Amphotericin B, therapeutic trials with this antibiotic were not successful and the patient died four months after operation. The post-mortem examination revealed a granulomatous Candida encephalitis, meningitis and ependymitis; in other organs, except the oral cavity, the oesophagus and nails no mycotic lesions were revealed.

"The described observation is a rare observation of candidiasis of the CNS, confirmed in vivo by cultivation and morphologically."

BERGMAN, F. **Reproduction and Pathogenicity of *Cryptococcus neoformans*.** *Sabouraudia*. Edinburgh. 1961, Jan., v. 1, Pt. 1, 34–40, 2 figs.

In the knowledge that animal passage increases the virulence of pathogenic bacteria, investigations were

instituted in order to ascertain if the strains of *Cryptococcus neoformans* would be similarly affected. 5 strains of *C. neoformans* were used for these experiments.

First the rate of reproduction of these strains was studied by estimating the proportion of budding organisms. It was found that budding began to decrease within 48 hours of inoculation and that it was not influenced by the age of the culture medium.

For virulence tests 0·02 ml. of a suspension of *C. neoformans* in physiological saline was injected intracerebrally into mice. No difference in virulence was observed between the different strains, and it was found that the survival time of the mice was inversely proportional to the dose of the suspension administered. Furthermore, the number of alternating passages through mice (from 42–57) and growth on culture media did not affect the mortality rate of the mice.

C. J. La Touche

STAIB, F. Vorkommen von *Cryptococcus neoformans* im Vogelmist. [***Cryptococcus neoformans* in the Faeces of Birds**] *Zent. f. Bakt.* I. Abt. Orig. 1961, v. 182, No. 4, 562–3.

FISCHER, E., HUTTER, R., LOEFFLER, W. & MÜLLY, K. Eine Mischinfektion menschlicher Lunge durch *Aspergillus fumigatus* und einen Streptomyceten. [**Mixed Pulmonary Infection with *Aspergillus fumigatus* and a *Streptomyces***] *Schweiz. med. Woch.* 1961, July 22, v. 91, No. 29, 864–6, 3 figs.

The English summary appended to the paper is as follows:—

"A human pulmonary infection with two micro-organisms found in a cyst is described. One of them is *Aspergillus fumigatus* Fres., the other, *Streptomyces albus* (Rossi-Doria emend. Krainsky) Waksman et Henrici, had been named *Actinomyces rangoon* Erikson and later on was changed to *Nocardia rangoonensis* Waksman et Henrici, both names based on a single strain."

ORIE, N. G. M., DE VRIES, G. A. & KIKSTRA, A. **Growth of *Aspergillus* in the Human Lung. Aspergilloma and Aspergillosis.** *Amer. J. Respiratory Dis.* 1960, Nov., v. 82, 649–62, 8 figs. [56 refs.]

After an introductory note a summary is given of the various clinical types met with in this pulmonary mycosis and is followed by a clinical and mycological survey of 22 cases observed by the first-named author of this article, as well as of 29 other cases.

In 44 of the patients concerned in this series infection by *Aspergillus* was associated with some other concomitant or pre-existent disease of a chronic nature. A table is given which includes, besides the name, sex and age of the patients and their occupation, their main clinical symptoms if any, on admission, and the chronic disease from which they were or had been suffering.

The pathogenesis and aetiology of aspergillosis are also discussed from various aspects, and methods of demonstrating micro-organisms in such cases are described and tabulated. Finally, some hints are given in regard to surgical treatment in localized forms of the disease.

C. J. La Touche

PECORA, D. V. & TOLL, M. W. **Pulmonary Resection for Localized Aspergillosis.** *New England J. Med.* 1960, Oct. 20, v. 263, No. 16, 785–7. [33 refs.]

Pulmonary resection was successfully performed on 3 patients with aspergilloma and resulted in their recovery.

2 of the patients were already suffering from tuberculosis and the third from silicosis.

The patient whose case is first described was a male hospital attendant aged 42 years. "Pulmonary decortication and resection of the left upper lobe were performed, without complication." A cavity contained therein was filled with much orange-brown necrotic debris and it was observed that a small bronchial communication to this cavity was present. It is thought that in this patient the cavity was of tuberculous origin and had become secondarily infected by *Aspergillus*. Positive cultures were not obtained.

The patient whose case is next described was a male farmer and miner aged 61 years who suffered from silicosis.

Pulmonary decortication and resection of the right upper and middle lobes was also performed. A large cavity measuring 6 cm. involved the interlobar fissure and enclosed "a large spongy, brown mass". This was made up of hyphae and "conidial elements compatible in morphology with aspergillus".

The patient concerned in the 3rd case described was a hospital attendant aged 39 years who was suffering from tuberculosis. X-ray examination of his chest revealed "residual pleurisy with calcification on the left and a large cavity in the left pulmonary apex".

Decortication of the visceral surface and wedge resection were carried out, and when the specimen so obtained was examined it was found to contain, as in the first case described, a cavity which was filled with necrotic orange-brown material. Again also it consisted of a mass of aspergillus hyphae and conidia, *sic*. Furthermore, it is assumed that in this case, likewise, the cavity was of tuberculous origin.

It is concluded that pulmonary resection is the treatment of choice in such cases.

C. J. La Touche

WASSERMAN, A. J., SHIELS, W. S. & SPORN, I. N. **Cerebral Mucormycosis.** *Southern Med. J.* 1961, Apr., v. 54, No. 4, 403–10, 6 figs. [22 refs.]

The clinical, pathological and morphological features of 3 further cases of cerebral mucormycosis [see this *Bulletin*, 1959, v. 34, 1006, 1118; 1960, v. 35, 125, 875] are described in this article.

The 3 patients concerned were: 2 Negroes, one aged 59 years, and the other 45 years; and a Negress aged 65 years. All 3 were, on admission, suffering from uncontrolled diabetes, and presented with symptoms and signs strongly suggestive of rhinocerebral mucormycosis.

In the first case treatment with amphotericin B and sodium iodide was instituted, but, despite this the patient's condition continued to deteriorate and he died on the 7th day after admission.

These drugs were also administered to the patient in the second case, but he likewise died a few days after admission. In his case a tooth extraction 18 days before his admission probably provided the portal of entry for the aetiological agent.

In the third case treatment consisted initially of chloramphenicol, tetracycline and ferrous sulphate. On the 40th day of admission, owing to progressive necrosis of the nasal bones surgical debridement was performed and large sequestra were removed. The patient was discharged on the 42nd day.

Hyphae characteristic of the Mucorales were demonstrated in biopsy material from the palate of the first patient, in necropsy material in the second patient and in necrotic material removed at operation from the nasal cavity of the third patient.

A table is included setting out the salient clinical and pathological features of 27 previously described cases as well as those observed in the 3 presently described cases.

C. J. La Touche

Communicable Diseases

The order in this section is:—**Tuberculosis; Venereal Diseases; General Infective Diseases; Other Communicable Diseases.**

For the bacteriology of these diseases see the section on Bacteriology below. For diseases conventionally regarded as tropical, readers are referred to the TROPICAL DISEASES BULLETIN

TUBERCULOSIS

GENERAL REGISTER OFFICE. **Studies on Medical and Population Subjects No 17. Mass Miniature Radiography. A Report on Three Years' Examinations in England and Wales 1955–1957** [HEASMAN, M. A.]. pp. x + 124, 3 diagrams. 1961. London: H.M. Stationery Office. [15s.]

The number of examinations made by mass miniature radiography units in England and Wales (population in 1956, 44,667,500) in the period 1955–57 was 10,607,860 (males 5,864,190 and females 4,743,670). There were 2 main groups, those referred by general medical practitioners and "public examinees" including hospital patients, armed forces, schoolchildren, contacts, workers, prisoners, residents of public institutions, general public volunteers, ante-natal patients, and inmates and personnel of mental hospitals.

Out of approximately 10½ million persons examined by X-ray 383,640 were recalled for further examination (36·2 per 1,000). The recall rate increased with age in both sexes and was higher in the group referred by general practitioners than in the other groups. Defaulter rates per 1,000 were 25·5 and 22·1 in males and females respectively in the doctors' group and 38·3 and 34·0 respectively in the others. A study of reasons for default would be of benefit in formulating the mass radiography policy.

Active pulmonary tuberculosis was found in 20,501 persons (1·93 per 1,000). This total represents 21·6% of all cases of pulmonary tuberculosis notified during the period. 14,836 of the cases were in public examinees (15·6% of all notifications). Among men the discovery rate tended to increase with age; the peak in women was reached at 20–24 years at which age the rates in all groups were higher than in males.

In all groups the incidence of active tuberculosis was higher among girls and young women than in males but above the age of 35 men showed a higher rate than women. In both sexes and at all ages the highest discovery rate was found in the group referred by doctors with the exception of men aged 45–64 in prisons and institutions for the destitute.

The rate in contacts was higher than in the remaining groups of public examinees which, of course, is not necessarily representative of the general population. The rate in mental hospitals was only slightly higher than among general public volunteers.

If it is assumed that active tuberculosis exists in the community in approximately the same proportion as it is found in factory workers and in general public volunteers (which is almost certainly an underestimate) then there are still over 50,000 and probably 75,000 persons over the age of 15 in England and Wales who have unrecognized active tuberculosis. This well-founded estimate will banish complacency.

Active disease was found more frequently among persons not previously examined by X-ray. At all ages in males the rates were 2·6 in those not previously examined and 1·15 in those previously examined and in females 1·89 and 1·04 respectively.

Cancer of the lung was found in 4,958 persons, representing less than 10% of the figure for all deaths from this cause during the period. It appears that mass radiography contributes little towards any reduction in mortality from lung cancer.

All persons examined were asked to state their occupation, and the information was analysed in respect of tuberculosis and cancer of the lung. For males in all groups there was a clear social class gradient with tuberculosis being found more frequently among the less skilled workers. This trend was also seen in women but less clearly. A group of men who seemed to be a

moderately high risk of contracting tuberculosis were those coming into contact with large numbers of people in their work, for example shop assistants, waiters, barmen, barbers and bus conductors. It may be that there is a certain selection of the less fit workers for such jobs but there is also the possibility that since tuberculosis is an infectious disease contact with many different members of the public may carry a greater risk of infection than exists for those who meet relatively few persons in the course of their work. The risk is, however, very small.

2 occupations in which women predominate were specially examined, *viz.*, nurses and laundry workers. There was no excess incidence of tuberculosis in either group.

The number of cases of lung cancer in women was so small that the social class or occupational analysis was restricted to men. Findings in the 5 social classes showed no real gradient. In Social Class II (intermediate occupations) the standardized abnormality ratio was significantly below normal and in Social Class V (unskilled occupations) significantly above normal.

H. G. Calwell

ELWOOD, P. C. **Tuberculosis in a Common Lodging-House.** *Brit. J. Preventive & Social Med.* 1961, Apr., v. 15, No. 2, 89–92. [12 refs.]

The common lodging house chosen for this study has 369 beds for the accommodation of men in Belfast [population about 450,000] and is situated in the smallest and most densely populated of the city's wards where there is a high incidence of tuberculosis.

During the period 1950–59, 36 cases of pulmonary tuberculosis were notified among the inmates which was equal to a yearly average rate of 9·8 per 1,000, assuming the lodging house to have been always full which it usually was. The average annual notification rate for the rest of the ward during the same period was 1·7 per 1,000 males of all ages.

Age-specific notification rates could not be calculated for the lodgers but the rate of 9·8 was very much higher than those for elderly men in the rest of the ward—2·8 in men aged 40–60 and 3·2 in men aged 60 and over.

A significantly higher proportion of lodgers than of other men had advanced disease at the time of notification, and the proportion of lodgers found to be excreting tubercle bacilli was also significantly higher.

The fate of the 36 patients at the end of 1959 was recorded: 13 had been discharged from hospital with inactive disease, 13 had died of tuberculosis, 6 had left hospital against medical advice, 1 had been lost sight of after diagnosis, and 3 were in hospital receiving treatment.

The spread of tuberculosis in the surrounding community was studied by analysing the spatial pattern of notifications. Adult males in 6 immediately adjacent streets provided an excess of cases while no such significant difference was seen in notifications from females. There was evidence that the excess was not due to a higher proportion of elderly males in the adjacent streets than elsewhere in the ward.

The high incidence of open tuberculosis and the uncooperative behaviour of the lodgers with tuberculosis create special problems in the community concerned. The author regards it as unlikely that spread occurs only within the lodging house. It probably also takes place outside, especially in places where males predominate; public houses and betting shops are considered to be the most likely places.

H. G. Calwell

LOTTE, A. & PERDRIZET, S. Résultats d'une enquête sur la tuberculose pulmonaire de l'adulte. **[Results of an Enquiry into Pulmonary Tuberculosis in Adults in France]** *Bull. Inst. Nat. Hyg.* Paris. 1960, Nov.–Dec., v. 15, No. 6, 921–52, 1 fig.

This paper is, in effect, a report based on 5,000 cases of pulmonary tuberculosis in adult patients in France, and the efficiency of the services provided for their care and supervision. Two-thirds of the patients investigated were suffering from relatively recent tuberculosis, *i.e.* their symptoms had not existed for more than 5 years (one-third of these had had the disease for less than 1 year). The remaining one-third of the patients had tuberculosis of a chronic type or had had a relapse of previously quiescent disease.

Their conclusions are summarized as follows. There is insufficient radiological cover by routine mass X-ray examination; one-quarter of the sufferers had not been summoned for this purpose before the onset of their disease, and another quarter had only had occasional examinations. 12% of the chronic cases were not known at the chest clinics before the date of this enquiry. The follow-up of patients suffering from tuberculosis was very incomplete; 3% of patients with acute disease, 12% with relapse, and 8% with chronic tuberculosis were not under observation. The number of patients whose treatment was either very sketchy, or who received no treatment at all, was considerable; there was a total absence of treatment in 8% of the patients with chronic disease, of correct treatment in 13% of new cases, and of about 40% in those patients who were suffering from relapse. Correct treatment was not promptly instituted after diagnosis; the delay exceeded one month in one-third of the patients, and 3 months in 10% of these. Treatment was also given to a considerable number of patients by physicians who had no special knowledge of tuberculosis. These conclusions are supported by a large number of statistical tables.

The social environment, with its economic and psychological factors, is regarded as very important in the campaign for control of tuberculosis.

F. H. Young

ROCHAT, P. Tuberculose et personel enseignant dans le Canton de Vaud 1930–1960. **[Tuberculosis in Schoolteachers in Vaud, Switzerland, 1930–60]** *Schweiz. med. Woch.* 1961, July 15, v. 91, No. 28, 819–25, 8 figs.

The author received reports of tuberculosis in schoolteachers whose disease was sufficiently severe to keep them off work long enough to draw special sickness benefit.

During the period 1930–42, 30 teachers were reported as having tuberculosis (1·3% of the total number of

teachers); 16 were men and 14 women. Omitting 2 student teachers and 4 re-employed pensioners there were 24 teachers aged between 21 and 46 years. 6 of these died of the disease (25%), 17 (71%) recovered and remained well for at least 15 years, 1 recovered but had been well for only 5 years, 12 resumed duty and 6 were unfit for further teaching.

As a result of the above findings the author recommended that every official medical examination of a teacher should include X-ray examination. This recommendation was accepted. He also advised that school medical officers should have the right to demand an X-ray examination of any teacher whose health was below normal or in whose class 2 or more cases of primary tuberculosis had occurred. This requirement did not become obligatory until 1956. His third recommendation was that teachers should be X-rayed routinely every 3 or 5 years. X-ray examination every 3 years was made compulsory in 1948. Finally he advised that if BCG vaccination was reported on favourably from other countries it should be used in adolescents in Switzerland. This measure was introduced in 1949.

During the period 1943–60, 20 teachers were reported to have tuberculosis (1% of the total); 10 were men and 10 women. 11 cases occurred before 1949 and 9 after 1949 in 6 of which the diagnosis was made on routine X-ray examination. 6 of those with pulmonary tuberculosis were found to be affected during their training period or in the first year of service, 13 with pulmonary tuberculosis were aged 23 to 48 years, and 1 teacher had renal as well as pulmonary tuberculosis.

1 of the 13 with pulmonary tuberculosis died, 4 gave up teaching and 8 recovered of whom 6 remained well for at least 6 years, 1 went on pension in good health and 1 left to get married.

Members of the public are well aware of the danger to children of a tuberculous teacher, and on occasion objection has been taken by parents to the return to duty of a teacher even after she had been certified free from infection.

In Rochat's opinion the anti-tuberculosis measures at present in use in the schools in the Canton of Vaud are sufficient. *H. G. Calwell*

Bognár, E. A tbc. morbiditás alakulása az iskolai szürövizsgálatok tükrében. **[The Course of Tuberculosis Morbidity on the Basis of School Survey Investigations]** *Népegészségügy*. Budapest. 1961, v. 2, No. 2, 43–7, 64, 1 chart. German summary.

The author reports the results of mass radiological surveys for the detection of tuberculosis infection carried out on 192,297 children aged 3–18 years in Budapest between 1933 and 1958. 518 (0·68%) active cases of tuberculosis were found among 76,332 children examined between 1933 and 1941, 432 (1·04%) among 40,599 examined in 1945–50, and 91 (0·12%) among 75,366 examined in 1950–58. The records for the 1941–46 period had been lost. The annual number of active cases detected has shown a considerable decline in recent years, falling from 16 of 10,100 children examined (0·15%) in 1953–54, to 5 of 9,328 (0·032%) in 1957–58.

D. J. Bauer

Bordas, J. & Mistrot, P. La tuberculose chez l malgache. **[Tuberculosis in Madagascar]** *Méd Trop.* Marseilles. 1961, May-June, v. 21, No. 3 241–50.

During the period 1949–59 there was a continuou increase in the number of patients receiving treatment fo tuberculosis in Madagascar. This may represent a tru increase in the incidence of the disease or it may b only the result of more patients seeking treatment. factors are relevant, *viz.* the development of medica facilities, health education, the efficacy of modern treat ment in prolonging the lives of tuberculous patients, an the economic development of the country which is leadin to increased urban populations with unhygienic condi tions, malnutrition and alcoholism.

The majority of the inhabitants still regard tuberculosi as something of which to be ashamed. They are there fore slow to seek advice at tuberculosis dispensaries Early diagnosis is exceptional, and the absence of an scheme of social security causes breadwinners to stay a work as long as they can.

In the authors' experience patients show grea resistance to co-operation with the doctor, many refus to stop work and conversely many who have recovere sufficiently to resume work are unwilling to do so. [I this peculiar to inhabitants of Madagascar?]. Man patients seek the advice of practitioners who treat them without X-ray or bacteriological examinations, wh dispense insufficient chemotherapy and dismiss them a cured when only a fleeting improvement has bee experienced.

Examination of the records of 1,100 patients showe that 47·2% were men, 26·4% women and 26·2% children Men attend the dispensaries more readily than women 814 (74%) had pulmonary tuberculosis of whom 3% were below the age of 15 years.

The disease was unilateral in 35·9%, bilateral in 63·9% and cavities were present in 75·3%. The tubercle bacillu was found on direct examination of sputum or gastri washings in 57·4% and in the majority of the remainde by culture or animal inoculation.

The mean period of stay in hospital was 4–6 months Ambulatory treatment was intended to be given for a least 8 months in cases of primary infection and fo 18–24 months in cases of pulmonary tuberculosis. N limit was placed to the duration of treatment of patient with chronic disease even in the presence of dru resistance. 98·1% of the patients treated received strepto mycin and isoniazid or these drugs with PAS. Othe forms of treatment used were artificial pneumoperitoneun (25·6%), artificial pneumothorax (10%) and surgica operation (2·7%).

The proportion of patients with positive sputa fe from 57·4% on diagnosis to 6·3% after treatment. Cur was thought to have been effected in 54%, and i addition 21·4% were much improved. Ambulatory treat ment was unsatisfactory because of the high defaulte rate although many of the defaulters returned later t resume treatment.

Prolongation of life in cases of chronic and uncure disease adds to the pool of infection and largely nullifie the excellent results obtained in patients whose diseas has responded to treatment. In these circumstances th

authors recommend compulsory routine mass X-ray examination and mass BCG vaccination. *H. G. Calwell*

INDIAN COUNCIL OF MED. RES. SPEC. REP. SER. NO. 34. **Tuberculosis in India. A Sample Survey, 1955–58.** pp. iii + iv + 121, illustrated. [12 refs.] 1959. New Delhi.

In order to obtain some reasonable data on which to form a plan for the control of tuberculosis in India, a special sample survey was carried out between the years 1955 and 1958. This report is an account of how the survey was carried out and the conclusions drawn from it. Six independent zones were surveyed by sampling; a single mass miniature X-ray film was taken, and sputum or laryngeal swab culture was made in all cases where the film suggested the likelihood of pulmonary tuberculosis.

The number of persons examined by X-ray was 290,758. The population included in the zones constituted 40% of the population of the Indian Union. About 88% of the selected sample of the population were actually examined by X-ray. Bacterial examinations were carried out on approximately 82% of those selected for this examination because their X-rays showed abnormality. Active and probably active tuberculosis varied from 13 to 25 per 1,000 of the population in the zones selected. 2 to 8 per 1,000 were bacteriologically positive. Little difference in the prevalence rate was shown in the cities, towns and villages; especially in the older age-groups the prevalence rate was lower for females than males. Very little minimal disease was discovered. In most of those diagnosed as having active or probably active disease the lesions were moderately advanced.

The people seem to have been extremely co-operative; a considerable proportion of those failing to be X-rayed were missed because of their absence from home.

The survey was carried out under special circumstances to meet a special need, and the facilities at the disposal of those taking part were considerably greater than can usually be expected. From the figures obtained in the survey it is estimated that about 5 million persons in India are suffering from pulmonary tuberculosis, and this gives some idea of the magnitude of the problem. *F. H. Young*

PALLASKE, G. **[The Pathological Anatomy and Pathogenesis of Spontaneous Tuberculosis in Animals.]**

This book was reviewed on p. 823.

RAMAKRISHNAN, C. V., ANDREWS, R. H., DEVADATTA, S., FOX, W., RADHAKRISHNA, S., SOMASUNDARAM, P. R. & VELU, S. **Influence of Segregation of Tuberculous Patients for One Year on the Attack Rate of Tuberculosis in a 2-Year Period in Close Family Contacts in South India.** *Bull. World Health Organization.* Geneva. 1961, v. 24, No. 2, 129–48, 3 figs.

This paper is an extension of one which reviewed a number of cases of tuberculosis occurring in the contacts of patients treated at home and at sanatorium under the same chemotherapy regime [this *Bulletin*, 1960, v. 35, 134]. The authors investigated the attack rate of tuberculosis during the first 2 years of a 5-year follow-up. The main comparison was between 256 contacts of 75 patients treated at home, and 274 contacts of 73 patients treated in sanatorium, in families of which the only infectious member was the index case. During the first year active tuberculosis developed in 27 contacts, namely, 9 (3·5%) home contacts, of whom 6 were initially tuberculin negative, and 18 (6·6%) sanatorium contacts, of whom 6 were initially tuberculin negative.

There were no cases among the initially negative tuberculin contacts in either group in the second year, but there were 3 (1·9%) in the initially tuberculin positive home contacts, and 4 (2·4%) in the corresponding group of sanatorium contacts. Of the 34 subjects who developed tuberculosis in a 2-year period, 17 were under the age of 5 years, and 24 under the age of 10 years. 18 (53%) of the cases arose during the first 3 months. These have all been attributed to infection by the index case before the start of treatment, since they included all the 6 cases in initially tuberculin negative sanatorium contacts who had been immediately segregated from the index case. Serial tuberculin tests did not give evidence of more tuberculous infection in the home than the sanatorium contacts. The attack rate of tuberculosis in the contacts of patients treated at home with effective chemotherapy was no greater than the contacts of patients segregated in sanatoria. It is concluded that the major risk to contacts in this study resulted from exposure to the index case before diagnosis. *F. H. Young*

DEVADATTA, S., ANDREWS, R. H., ANGEL, J. H., BHATIA, A. L., FOX, W., JANARDHANAM, B., RADHAKRISHNA, S., RAMAKRISHNAN, C. V., SUBBAIAH, T. V. & VELU, S. **Progress in the Second and Third Years of Patients with Quiescent Pulmonary Tuberculosis after a Year of Chemotherapy at Home or in Sanatorium, and Influence of Further Chemotherapy on the Relapse Rate.** *Bull. World Health Organization.* Geneva. 1961, v. 24, No. 2, 149–75, 1 diagram. [28 refs.]

This study deals with 126 patients out of 130 in the main analysis of the previous report who were classified as having bacteriologically quiescent disease at the end of the first year. They were divided into 8 groups, those categorized as " isoniazid " continued with their isoniazid treatment, PAS being omitted, whereas the remainder received calcium gluconate as a placebo. Evidence was obtained that about 20% of both groups failed to take their treatment regularly. In the second year 5 patients in the calcium group, and 3 in the isoniazid group, relapsed bacteriologically, and one isoniazid patient died. Of the isoniazid group, 60 were allocated to calcium gluconate (30 patients) or to isoniazid (30 patients) for the 3rd year. Of these 27 had been treated at home in the first year, and 33 in the sanatorium, 19 having residual cavitation, and 41 no residual cavitation.

At the end of the 3rd year there was relatively little difference between the progress of patients in the various sub-groups. 6 (10%) of 61 patients who received 1 year

of chemotherapy, relapsed bacteriologically, as compared with 3 (5%) of the 65 who received 2 or 3 years of chemotherapy. 3 (5%) of 57 patients treated at home in the first year relapsed, as compared with 6 (9%) treated in the sanatorium. 4 (10%) of patients with the open negative syndrome relapsed, as compared with 5 (6%) of 84 in the non-cavitated series.

The authors conclude that there was relatively little difference between the patients whose disease attained quiescence following a year of domiciliary therapy, and those whose disease attained quiescence following a year's treatment with the same combination of drugs in a sanatorium. There was no evidence that a second or third year's treatment with isoniazid alone gave better results than a year of chemotherapy followed by careful observation of the patient, and further chemotherapy if relapse occurred. Patients with residual cavitation at one year had a slightly higher relapse rate than those without residual cavitation.

Place of treatment during the first year	Cavitation status at the end of the first year	Treatment during the second year	Number of patients
Home	Cavitated	Calcium	11
		Isoniazid	10
	Non-cavitated	Calcium	18
		Isoniazid	18
Sanatorium	Cavitated	Calcium	8
		Isoniazid	13
	Non-cavitated	Calcium	24
		Isoniazid	24
		Total	126

F. H. Young

KERTAY, N., VÉN, F. & HORVÁTH, J. A *Mycobacterium tuberculosis* bovin típusának elöfordulása az emberek gümökóros megbetegedésében, a kiskörösi járásban. [**The Occurrence of *Mycobacterium tuberculosis* of Bovine Type in Human Cases of Tuberculosis in the Kiskörös District**] *Népegészségügy*. Budapest. 1961, Feb., v. 42, No. 2, 41–3, 63. [21 refs.] German summary (5 lines).

The authors have investigated the incidence of bovine tuberculosis in a district in Hungary inhabited by 66,000 persons, 750 of whom were registered as suffering from tuberculosis. Foci of the infection among cattle farms were detected by clinical examination of the animals and by tuberculin tests, and persons living on farms with infected animals were examined clinically and radiologically. Cattle infected with bovine tuberculosis were found on 608 (13·9%) of 4,417 farms investigated, and of 3,642 persons living on the affected farms who wer investigated 174 (4·7%) were suffering from tuberculosis including 6 cases not previously notified. 87 strains o tubercle bacillus were isolated from the sputum of thes persons, 71 (81·8%) of which were of human and 1 (13·7%) of bovine type. *D. J. Bauer*

WATSON, W. N. B. & WILLISON, Jean. **Tuberculin Testing of School Children.** *Med. Officer*. 1961 Aug. 4, v. 106, No. 5, 83–7. [10 refs.]

Tuberculin testing of schoolchildren as a group is used to detect dissemination of infection when a member o the school staff or a pupil has been found to have infectious tuberculosis, or to select reactors not at specia risk and their family contacts for X-ray examination, o to select candidates for BCG vaccination, or, finally, to obtain an index of infection in the general community The test has been used for all these purposes in Edinburgh. The method of intracutaneous injection was used from the initiation of the scheme in 1954 until 1957 when the Heaf method was introduced.

Since 1954 it has been the practice when infectious tuberculosis has been notified in school personnel or a pupil to test classes believed to have been at risk. From 1954 to 1960, 2,263 pupils were tested in connexion with 37 infectious cases. No dissemination of the disease was found in 36 instances, but in one a pupil was found to have infected 11 (61%) of her 18 classmates of whom 4 had active tuberculosis requiring treatment. No other source of infection was found nor were any children outside the one class infected.

In 1957 it was decided to offer tuberculin testing to 5-year-old children in 36 schools and to some 9 years old. The family contacts of all unvaccinated reactors were invited to attend for X-ray examination. In the younger children 32 (1·3%) of 2,597 were natural reactors and 1 had active tuberculosis; in the older group 18 (7%) of 259 reacted naturally and none was found to have active tuberculosis.

In 1958 testing was also offered to all pupils (5–12 years) in 8 schools and 4,067 were tested. There were 199 natural reactors (4·9%) of whom 3 had active tuberculosis. 454 (81%) of 560 home contacts were X-rayed and none found to have active tuberculosis.

In 1959, 3,664 pupils aged 5–12 years were tested in schools not previously surveyed. 158 (4·3%) were natural reactors and 3 were found to have active tuberculosis 2,231 pupils were retested in schools previously surveyed of whom 41 were found to have undergone tuberculin conversion since the previous year and 4 to show a higher degree of sensitivity. None of these had active tuberculosis. No active disease was found in 207 home contacts X-rayed.

During the period 1954–60, 31,481 pupils in their 14th year were tested as candidates for BCG vaccination 6,052 (19·2%) were reactors and 25,429 were vaccinated The effect of vaccination was assessed by subsequent notifications of tuberculosis. 6 (0·3 per 1,000) of the vaccinated children and 44 (8·4 per 1,000) of the reactor developed tuberculosis. There was no unvaccinated non reactor control group owing to the high response rat for vaccination. The high incidence of tuberculosis i

the natural reactors causes the authors to emphasize the importance of repeated annual X-ray examinations of reactors. [Would it not be sufficient to afford this supervision to those displaying hypersensitivity?]

The decrease in the reactor rate in the 14-year-old children from 30·5% in 1954 to 13% in 1960 shows accurately the increasing efficiency of tuberculosis control in Edinburgh. During almost the same period the tuberculosis death rate fell from 19 per 100,000 to 4 in 1959 and the notification rate from 170 to 59.

H. G. Calwell

ROYAL NETHERLANDS TUBERCULOSIS ASS. **Tuberculin Conversion and Reversion** [GRIEP, W. A.]. *Proc. Tuberculosis Res. Counc.* 1960, No. 47, 5–23, 4 figs.

In order to study tuberculin conversion and reversion, data were collected on tuberculin reactions in 432 students in Amsterdam with an induration of 10 mm. or less to the Mantoux test with 10 TU of PPD. Students who had never been vaccinated with BCG were repeatedly tested at intervals of 1 year. The results of 3 to 7 subsequent reactions from those who on all occasions reacted with less than 11 mm. were analysed.

The series could be divided into several groups. Reactions of 6 mm. or over were considered as positive e.g. 7-7-7-7-7-8-6, and those of 5 mm. or less as negative *e.g.* 0-0-1-0-4-0-0. There were however series which could not be placed in either of these groups and represented either conversion or reversion. Examples were:

consistent conversion: 3-9-8-7-10 mm.
consistent reversion: 7-8-5-0-0-3 mm.
inconsistent conversion: 1-6-1-3-4-2-2 mm.
inconsistent reversion: 6-1-7-4-1-1 mm.

The probability of "positive" standing for "infected" and "negative" for "uninfected" was thought to increase with the passage of the years. It also appeared that a consistent conversion was more likely to represent tuberculous infection as the series grew longer, but this was not always certain as for example in the 2 series: 1-8-8-6-6-2-3 and 4-7-10-9-9-2-1 mm.

The instability of low-grade tuberculin sensitivity is probably the result of several factors including experimental errors such as lack of potency of the tuberculin dilution used, adsorption of tuberculin to the wall of the container, accelerated reaction, errors in reading or recording and faulty injection technique. Infectious diseases and pregnancy may lower sensitivity temporarily, non-specific infections may cause low-grade sensitivity and BCG vaccination, forgotten and not mentioned by the subject, may be responsible for inconsistent sensitivity.

Other series of tests, of which details are given, showed that the Mantoux test often fails to separate clearly the infected from the uninfected. In a series of 346 children recently exposed to tuberculous infection Mantoux tests with 5 TU repeated after 6 weeks gave 5 "reversions" and 35 "conversions". Tuberculous lesions were found only in the children whose reactions showed a marked increase in the diameter of induration. These reactions measured 11–17 mm. The author proposes that the term "conversion" should be used only when the "negative-positive" line is crossed with at least 4 mm. of increase.

H. G. Calwell

ROYAL NETHERLANDS TUBERCULOSIS ASS. **Intracutaneous Reactions with Tuberculins prepared from *Myc. tuberculosis humanum* and from BCG** [GRIEP, W. A. & BLEIKER, M. A.] *Proc. Tuberculosis Res. Counc.* 1960, No. 47, 24–33, 5 figs.

There are no definite criteria by which it can be determined whether tuberculin sensitivity in a given case is due to vaccination with BCG or to infection with virulent tubercle bacilli. The tuberculin test is therefore of little use as an epidemiological or diagnostic tool in a vaccinated population.

Double tests with ordinary mammalian tuberculin and tuberculin prepared from non-specific mycobacteria are already being used to investigate "non-specific" tuberculin sensitivity, and the authors have used this method to try to differentiate by means of a double test with ordinary and BCG tuberculin between persons vaccinated with BCG and not superinfected with virulent tubercle bacilli from persons vaccinated and later superinfected. The theoretical basis of the trial is that infection with a given variety of mycobacterium should cause a greater degree of sensitivity to homologous than to heterologous tuberculin.

454 young men of whom 68 had previously been vaccinated with BCG were given 2 Mantoux tests simultaneously, 1 on either arm with 1 TU of PPD-Rt XXIII and 1 TU of BCG tuberculin. Tween 80 had been added to both tuberculins to prevent adsorption of the tuberculo-protein to the glass of the containers. The results were plotted according to the size of the reaction. There were no indications of 3 separate groups, which meant that the double test failed to identify those vaccinated with BCG and superinfected with virulent tubercle bacilli. It was found that those showing tuberculin sensitivity reacted somewhat more strongly to human than to BCG tuberculin.

Other studies were made, none of which yielded satisfactory identification of the various groups. There were however differences in the sizes of reactions to the double tests which led the authors to assume "that it may be possible to identify post-vaccinal from post-infectious tuberculin allergy, once more specific tuberculins, or tuberculin fractions, will be obtained." *H. G. Calwell*

FRIEDLANDER, F. C. & PROZESKY, L. **The Tuberculin Test. Observations on Non-European Infants under the Age of One Year.** *Med. Proc.* Johannesburg. 1961, June 17, v. 7, No. 12, 232–6, 3 figs. [15 refs.]

During 9 months of 1960, 354 non-European infants under 1 year of age, most of them Bantu, were admitted to a general hospital in Natal, South Africa. After excluding 174 who died within 48 hours, or were under 6 weeks of age, or had not been tuberculin tested, or had already been vaccinated with BCG, there remained 180 who received the Mantoux test with 250 TU of PPD.

30 of the 180 infants were considered to have tuberculosis, and in addition 4 died of tuberculosis before they could be tested so that an apparent total of 34 infants had tuberculosis (9·6% of all admissions in the period).

The criterion of a positive reaction was 8 mm. of induration at 48–72 hours; 15 of the 30 infants in whom tuberculosis was diagnosed were classified as reactors and 15 as non-reactors. One of the non-reactors became a reactor after 4 months' specific treatment.

After reviewing the cases the authors concluded that the following points are of importance for the diagnosis of tuberculosis in the absence of a positive Mantoux reaction:

1. Failure to gain weight adequately after 4–6 weeks' feeding and repeated courses of antibiotics.
2. A stationary or falling level of haemoglobin.
3. A constant unexplained pyrexia in spite of treatment.
4. A pellagrinous type of rash unaffected by diet or vitamins.
5. Radiological evidence of pulmonary infiltration unaffected by antibiotics.
6. Response of all the above conditions to anti-tuberculous treatment.

The high non-reactor rate in this series of infants in whom the diagnosis of tuberculosis was not doubted by the authors is discussed. The demonstration of tuberculin sensitivity depends on the persistence of tuberculin at the site of injection for an adequate time and the availability there of a sufficient number of antibody-carrying cells (lymphocytes) [this *Bulletin*, 1955, v. 30, 485]. Tuberculin is removed rapidly whenever there is increased lymphatic absorption as in febrile and infectious disorders, hunger cachexia, exposure to bright sunlight and other irritants. Local factors preventing lymphocytes from reaching the site of the test such as vaso-constriction will depress the reaction as will factors depressing lymphocytes and lymphoid tissue such as cortisone or radiation with X-rays. The need for the utmost care in the execution of the test is emphasized.

A comprehensive list of the several factors which are known to depress the tuberculin reaction is given. Those which the authors believed to be at work in the present series were: the state of nutrition, anaemia, serious illness, the age of the infants (under 1 year), febrile and infectious disorders and perhaps (but unlikely) lack of potency of the PPD used. *H. G. Calwell*

Duboczy, B. O. & Brown, B. T. **Multiple Readings and Determination of Maximal Intensity of Tuberculin Reaction.** *Amer. Rev. Respiratory Dis.* 1961, July, v. 84, No. 1, 60–68. [36 refs.]

The course of the tuberculin reaction varies not only in different individuals but also in the same individual at different times, and therefore the single reading of the result of a test may lead to error in interpretation. The authors have studied the results of single and multiple readings in order to determine at what point the reaction reaches its maximum intensity.

The Mantoux test was used with 1, 10 and 250 TU of PPD. The diameter and height of induration were measured at 12, 24, 48 and 72 hours and also 5 and 7 days after injection. Absence of induration with or without erythema was interpreted as a negative result and induration of 5 mm. or less as doubtful.

Results were analysed for 308 patients with proved tuberculosis of whom 295 (95·8%) had pulmonary tuberculosis and 13 (4·2%) extrapulmonary. The disease was active in 237 (76·9%) and inactive in 71 (23·1%). In the patients with pulmonary lesions the disease was primary in 5 (1·6%), minimal in 26 (8·4%), moderately advanced in 139 (45·2%) and far advanced in 125 (40·6%).

The reaction to 1 TU was positive in 77·6% at 48 hours and in 82·5% with multiple readings throughout the 7-day period. 15 reactions reached the positive ranges only after 48 hours. 54 patients remained negative to 1 TU even with multiple readings. They were retested with 10 TU and 46 reacted at 48 hours whereas 48 were found to react with the multiple readings. The cumulative reactor rate to 10 TU at 48 hours was 97·4% and with multiple readings 98·1%. 6 patients who remained negative even with multiple readings were retested with 250 TU; 4 were reactors at 48 hours and all were reactors with multiple readings.

The distribution of maximal reactions according to the interval after injection of different doses of PPD was examined. None of the reactions had reached the maximum at 12 hours. At 24 hours 4·7% of reactions to 1 TU and 18·8% to 10 TU had reached the maximum. At 48 hours the rates were 56·7% and 62·4% respectively, at 72 hours they were 26·3% and 14·6% and at 5 days 11·1% and 2·1% respectively. 3 reactions to 1 TU and 1 to 10 TU reached the maximum at 7 days. In the 250 TU group there were only 6 patients and their reactions were evenly distributed at 12, 24 and 48 hours.

The time of maximal intensity showed some correlation with the activity and stage of the disease. It appeared that the reaction usually reached its maximum intensity sooner in patients with active than with inactive disease. Similarly the reaction usually reached its maximum earlier in the acute, sub-acute and progressive stages of the disease than in the chronic stage.

The percentage of slight reactions was higher with inactive than active disease, and medium and strong reactions occurred in about the same proportion in both groups. The size of the maximal reaction was however not definitely correlated with the stage or activity of the disease. *H. G. Calwell*

Lillehei, J. P. **Sputum Induction with Heated Aerosol Inhalations for the Diagnosis of Tuberculosis.** *Amer. Rev. Respiratory Dis.* 1961, Aug., v. 84, No. 2, 276–8, 2 figs. [Refs. in footnotes.]

"In patients suspected of, or undergoing treatment for, pulmonary tuberculosis who produced little or no sputum, tubercle bacilli were identified more often from sputum induced by inhaling a heated aerosol of 10 per cent glycerin and 10 per cent sodium chloride in water than from gastric washings."

SCHWARTZ, I. & SMALL, M. J. **Preliminary Studies in the Use of Superheated Saline Nebulization in the Bacteriologic Diagnosis of Pulmonary Tuberculosis.** *Amer. Rev. Respiratory Dis.* 1961, Aug., v. 84, No. 2, 279–80.

"The production of sputum by means of the superheated saline nebulizer is useful in the bacteriologic diagnosis of tuberculosis. This method is particularly valuable in patients who do not have a productive cough. The bacteriologic findings in sputum obtained by nebulization correlated well with those obtained by tracheal and gastric lavage. The average time necessary to produce a cough by the nebulization method in 85.4 per cent of the patients was three minutes."

DES PREZ, R. & BOONE, Irene U. **Metabolism of C^{14}-Isoniazid in Humans.** *Amer. Rev. Respiratory Dis.* 1961, July, v. 84, No. 1, 42–51, 8 figs. [26 refs.]

WEISS, W., HING HUA CHUN & FLIPPIN, H. F. **The Treatment of Pulmonary Tuberculosis with Isoniazid and Sulfadimethoxine: a Controlled Study.** *Amer. J. Med. Sci.* 1961, Apr., v. 241, No. 4, 448–53, 1 fig.

In view of the favourable reports from some sources of the value of sulphonamides in the therapy of pulmonar tuberculosis, it was decided to try sulphonamides plus isoniazid in a controlled study, and compare them with isoniazid and PAS. The Japanese have reported satisfactory results. They found that sulphisoxazole combined with isoniazid was as good as triple therapy in a small series of cases. *In vitro* examinations of various sulphonamides for their efficiency in the inhibition of *Mycobacterium tuberculosis* wild strains showed that all of 11 strains were inhibited by 50 μgm. per ml. or less of sulphisoxazole and sulphadimethoxine (Madribon). The latter in combination with isoniazid was tried on 20 patients and comparison was made with 20 other well matched patients who were treated with isoniazid and PAS. Sputum conversion took place in all but one of the 17 subjects who were treated with isoniazid-PAS for 4 months, whereas with the isoniazid-sulphadimethoxine group only 5 patients achieved a negative culture.

Radiological resolution equivalent to 30% or more took place in 11 out of the 20 patients treated with isoniazid and PAS as against 4 treated with isoniazid-sulphadimethoxine. In the isoniazid-PAS group 3 patients out of 16 with cavities showed disappearance of the cavitation within 3 months. In the isoniazid-sulphadimethoxine group, however, only one patient out of 15 with cavities showed cavity closure and this occurred during the fourth month of therapy. At the commencement of the investigation in the PAS groups 6 cultures grew in 0·2 μgm./ml., 3 in 1·0 μgm./ml., and 1 in 5·0 μgm./ml. isoniazid. In the isoniazid-sulphadimethoxine group 7 cultures grew in ·2 μgm./ml., and 3 in both 1·0 μgm./ml. and 5·0 μgm./ml.

In the isoniazid-PAS group drug resistance at various levels seemed to have no clinical significance since all cultures of these patients converted to negative at the end of 4 months. In contrast, 6 of the 7 patients with varying degrees of isoniazid resistance in the isoniazid-sulphadimethoxine group showed poor radiological results and their sputa failed to convert on culture.

Defining increased resistance as the occurrence of growth at two higher concentrations of isoniazid, none of the isoniazid-PAS cases showed increased resistance during therapy, but one of isoniazid-sulphadimethoxine cases did.

The authors conclude that in spite of the good results *in vitro* shown by the sulphonamides as regards tuberculostatic activity the clinical study showed very poor results, in fact only the non-cavity cases did well.

F. H. Young

PRIDIE, R. B. & STRADLING, P. **Management of Pulmonary Tuberculosis during Pregnancy.** *Brit. Med. J.* 1961, July 8, 78–9. [17 refs.]

Even before the introduction of antimicrobial treatment for pulmonary tuberculosis there was some doubt about the effect of pregnancy on the incidence of relapse. Since the advent of the drugs it has become more and more accepted that pregnancy has little effect on the fate of the patient with pulmonary tuberculosis. This paper provides considerable evidence in favour of this concept. 103 patients who had had pulmonary tuberculosis during the 5 years before conception were examined retrospectively. It was found that there was no significant difference between the age-groups in the various chemotherapy categories, and the various extents of disease had similar distribution. 28 patients had chemotherapy during pregnancy and none, either among those who had had chemotherapy initially or those who had not, relapsed. Among the patients who had no chemotherapy during pregnancy (75) there was a difference between those who had received chemotherapy for their original disease and those who had not. Of 24 who had initial chemotherapy, 3 relapsed, and 21 had no relapse. Of the 51 who had no initial chemotherapy, 17 relapsed, and 34 had no relapse. There was no difference in the relapse rate between patients who were rested and those who were not, but there were only 14 in the resting group so the number is too small to allow of valid conclusions. A further analysis of the relapses shows that the rate for mothers who breast-fed was only one-fifth of the rate for those who did not. However, this is not a valid association conclusion, because it is probable that only patients with apparently quiescent disease would be allowed to feed their infants.

The authors conclude that women with pulmonary tuberculosis which has shown any activity within the previous 5 years can safely undertake pregnancy and labour without relapse provided they receive adequate chemotherapy throughout the period of pregnancy. Provided that the patient is well, rest need not be prescribed. Routine ante-natal chest radiography is essential to discover tuberculosis early enough for adequate control.

F. H. Young

Berte, S. J., Tucker, E. B. & Angen, W. F. **The Identification of Tubercle Bacilli in 136 Surgically Resected Lung Specimens. The Influence of Preoperative Chemotherapy utilizing High-Dosage and Low-Dosage Isoniazid.** *Amer. Rev. Respiratory Dis.* 1961, May, v. 83, No. 5, 748–50.

The purpose of this study was to see whether there is any difference in the incidence of tubercle bacilli in resected lungs from patients treated with a high dosage of isoniazid as compared with those from patients on a low dosage of the drug. The high dose was given to 68 patients during 1957–59 and the daily dose was 10–20 mgm. per kgm.; in addition to this treatment the patients were given PAS and 100 mgm. pyridoxine, and some also received streptomycin. The low dose of isoniazid was also given to 68 patients, the daily dose being 300 mgm. and in addition 1 gm. of streptomycin was given every 3rd day; a few of these patients also received PAS. Tubercle bacilli were looked for in stained smears of caseous material from the resected lungs and in sections of the lesions. Homogenates were inoculated on to Löwenstein-Jensen and an oleic-acid-albumin medium, and also inoculated into guineapigs.

The results are analysed in several ways and presented in 5 tables. The sections were positive for acid-fast bacilli in 45 of the low-dose and in 53 of the high-dose cases as compared with 38 and 41, respectively, positive by smear. Positive cultures were obtained from 22 of patients on the low-dose and 23 on the high-dose, these figures comparing with 21 and 30, respectively, giving positive results in guineapigs. Bacilli were not demonstrated by either method in 8 patients on the low-dose and 9 on the high-dose. Of the patients who were operated on within 1–5 months of commencing treatment, the cultures and guineapig tests were negative in 47% of those who received the low dose of isoniazid and in 25% of those who received the high dose, but in those who were operated on within 6–12 months the percentages were 67·6 and 64·5, respectively.

The authors conclude that there is no significant difference in the effects of these 2 regimens on the recoverability of tubercle bacilli from resected lungs, that including the guineapig test increased by 12% the number of cases from whom viable bacilli were recovered, and that sections of the lesions are superior to stained smears of the caseous material for demonstrating the bacilli. *S. R. M. Bushby*

Domagk, G. Tuberkelbacillresistens och kemoterapi vid tuberkulos. [**Bacterial Resistance and Chemotherapy in Tuberculosis**] *Nordisk Med.* 1961, Aug. 3, v. 66, No. 31, 1077–80. [48 refs.]

The English summary appended to the paper is as follows:—

"In 1946—1947 the author and his collaborators were able to demonstrate anatomical healing of some forms of tuberculosis after treatment with thiacetazone (Conteben). Bacterial resistance may develop to aminosalyl, streptomycin, isoniazid and thiacetazone, but least often to thiacetazone. Isoniazid, which is the corner stone in all tuberculosis chemotherapy, has a considerable inhibitory effect, which explains why isoniazid therapy can be effective only with treatment for ½ year or more. To prevent the emergence of isoniazid-resistant strains thiosemicarbazones or sulphathiazole are given, most conveniently as Nicoteben, Eleudron or Cibazol. The value of combined treatment is that the different drugs act on different enzyme systems in the tubercle bacillus, which cannot survive a blocking of three enzyme systems essential to life. Primarily resistant strains emerge probably because of inappropriate or inadequate treatment. A truly scientific chemotherapy will not be achieved until the optimum forms of combination have been worked out by studies of the serum-concentration of different resistant strains."

Eidus, L., Greenberg, L. & Little, Elizabeth. **Methods of assaying Antituberculous Drugs in Biologic Material. I. The Chemical Assay of Alpha-Ethylthioisonicotinamide (TH 1314).** *Amer. Rev. Respiratory Dis.* 1961, July, v. 84, No. 1, 98–9. [Refs. in footnotes.]

VENEREAL DISEASES

Touraine, A. Incidence de l'homosexualité sur la morbidité vénérienne. [**Incidence of Homosexuality in Relation to that of Venereal Diseases**] *Rev. d'Hyg. et de Méd. Sociale.* Paris. 1961, July–Aug., v. 9, No. 5, 474–84. [Numerous refs.]

In this connexion granuloma inguinale, chancroid and lymphogranuloma venereum play a very small part though the last-named affected 10% of males in one series of cases. In recent years there has been a steady increase in gonorrhoea and infectious syphilis in many civilized countries and this appears to have coincided with an increase in homosexuality. Most of the early part of this article consists of quotations from the reports of various authors of the number of homosexuals found to be suffering from venereal disease.

That there has been an increase in homosexuality in the last few years seems certain [Kinsey's figures of 4·33% of males in the United States are convincing] and this appears in all strata of society; moreover excessive sex-impulse and promiscuity are characteristics of homosexuals. Seeing that increased homosexuality and

increase of both gonorrhoea and early spyhilis have occurred simultaneously in many countries, it is hard to believe that the two are not associated.

The struggle against homosexuality is mainly a sociological matter and both the medical profession and public authorities should be induced to realize the danger.

[This and similar articles deal only with male homosexuals; it would be interesting to have some figures concerning females among whom homosexuality may, perhaps, be even commoner.] *T. E. Osmond*

TAITZ, L. S., ISAACSON, C. & STEIN, H. **Acute Nephritis associated with Congenital Syphilis.** *Brit. Med. J.* 1961, July 15, 152–3, 3 figs. on pl. [13 refs.]

Judging from the paucity of the literature acute nephritis associated with congenital syphilis is rare.

These authors describe 2 cases. The first patient, an African male child aged 6 weeks, was pale, irritable and showed generalized oedema; liver and spleen were enlarged as were both kidneys; urine showed albuminuria, red cells and granular casts. Blood urea was 95 mgm. per 100 ml. and haemoglobin was 10·7 gm. per 100 ml. and white cells were 52,000 per cmm.

There was periostitis of the femora and mother and child both had positive VDRL tests.

Treatment with penicillin brought about temporary improvement but the child died on the 10th day. At autopsy kidneys were found enlarged and showed marked changes in the glomeruli typical of acute glomerulonephritis.

The second patient was an African female aged 2 months showing swelling of the face and feet and enlarged liver and spleen; urine contained albumin, blood and casts. VDRL was positive in the mother but negative in the child. Knees showed metaphysitis and periostitis. Treatment with penicillin brought about clinical improvement and blood urea fell from 26 mgm. to 18 mgm. per 100 ml. After 25 days there was clinical recovery though periostitis persisted. *T. E. Osmond*

PAPAIOANNOU, A. C., ASROW, Gertrude G. & SCHUCKMELL, Natalie H. **Nephrotic Syndrome in Early Infancy as a Manifestation of Congenital Syphilis.** *Pediatrics.* Springfield, Ill. 1961, Apr., v. 27, No. 4, 636–41, 3 figs. [30 refs.]

A case of a 10-week-old male child is reported. Signs began at the age of 6 weeks, ascites, hepatosplenomegaly and oedema of various parts of the body; there were proteinuria, haematuria and cylindruria. The electrophoretic pattern was abnormal (that of a normal infant, of a nephrotic infant and of this patient before and after treatment is shown in a figure), albumin, alpha and gamma globulins decreased, beta-globulin was normal but alpha$_2$-globulin increased. Serum tests for syphilis including Kahn, Kolmer WR, and TPI were all positive; the Kahn test of the cerebrospinal fluid was positive but the colloidal gold curve was normal. X-rays showed osteochondritis and periostitis of radius and ulna. The mother was seropositive but the father and a sibling were seronegative.

Treatment with 1·2 mega units of procaine penicillin G daily for 10 days brought about marked improvement, and a second course established apparent cure [the date of the last observation is given as "present time"].

Most of the abnormalities cleared in about a month but positive serum tests for syphilis persisted for over 6 months [apparently a second TPI test was not done]. Full details of progress, showing gradual return to normal of lesions, and of various tests, are set out in a figure. [The haemoglobin (gm. per 100 ml.) in the table is given as 72·5.] *T. E. Osmond*

RIDET, J. & ODDOU, A., with the technical collaboration of M. A. BAMBARA. Une nouvelle technique sérologique des tréponématoses de pratique courante: le "Rapid Plasma Reagin Test". [**The Rapid Plasma Reagin Test in Treponematoses**] *Méd. Trop.* Marseilles. 1961, May–June, v. 21, No. 3, 282–90.

This test (RPRT) [see this *Bulletin*, 1960, v. 35, 994] is carried out as for the VDRL or Kline test with plasma obtained by adding an anticoagulant such as heparine, potassium oxalate or potassium sequestrin to the blood; the antigen is prepared by centrifuging VDRL antigen suspension at 2,000 r.p.m. for 15 minutes and adding to the precipitate an equal volume of choline hydrochloride in merthiolate [thiomersal]; this latter is made up by adding 3 volumes of 0·0133% merthiolate to 1 volume of 40% choline hydrochloride.

Detailed results of testing specimens from patients suffering from trypanosomiasis (18), leprosy (30), various other diseases including syphilis (treated and untreated), and yaws (6), and of mass testing are set out; reactions employed included the Kline, VDRL, Kolmer and Reiter [? Reiter Protein complement-fixation test].

From the results of these it is concluded that RPRT is equal in sensitivity to the Kline and superior to other reactions, and remains positive as long as complement-fixation reactions; its specificity is difficult to assess.

For mass testing in the field the RPRT is superior to most other tests; it is quicker, cheaper and more economical of staff and material, but the authors stress that an electric centrifuge is essential.

[To say that its sensitivity is equal to that of the Kline does not help much; what one wants to know is whether it is reactive in trypanosomiasis, leprosy and other tropical diseases in the absence of syphilis or yaws.] *T. E. Osmond*

WIDELOCK, D., PORTNOY, J., TRUELOVE, J., REYNOLDS, Anna D. & VANDOW, J. **The USR Test as a Screening Test in a Public Health Laboratory.** *Pub. Health Rep.* Wash. 1961, May, v. 76, No. 5, 447–52. [10 refs.]

The unheated serum reagin test (USR) was performed on slides with paraffin rings 14 mm. in diameter by using 0·06 ml. of serum and 0·02 ml. of antigen. 100,000 tests for syphilis were carried out by USR and VDRL in

parallel; those reacting with either were submitted to the Kolmer complement-fixation test and some to the Reiter protein (RPCF) and tpcf-50 complement-fixation tests. Details showing results, agreements and disagreements are set out in 6 tables. There was 98·2% agreement between USR and VDRL tests on the 100,000 sera: on 1,885 sera from patients in various stages of syphilis there was agreement in 92% though somewhat less in latent than in clinical cases; both tests were positive in 62% of cases diagnosed as biologically false positive. When the diagnosis was syphilis and USR positive, Kolmer was positive in 74·5%, RPCF in 71·1% and tpcf-50 in 71·2%.

It is concluded that USR will detect at least as many reactions as VDRL, while being cheaper and easier to perform, and that it may well be used as a screen test. RPCF and tpcf-50 gave somewhat variable results. The procedure recommended therefore is to use USR as a screen test and retest all reacting sera by VDRL (quantitative), Kolmer CFT and RPCF: if a more confirmatory test is required (and TPI is not practicable) a test with virulent spirochaetal antigen, such as the fluorescent treponemal antibody test (FTA), should be used.

T. E. Osmond

Ridet, J. & Oddou, A. L'antigène de Reiter et la sérologie classique dans les tréponématoses exotiques. **[The Reiter Antigen and Classical Serological Tests in Treponematoses in the Tropics]** *Méd. Trop.* Marseilles. 1961, Mar.–Apr., v. 21, No. 2, 169–71.

Examination of 4,849 sera in the biological laboratory of the Muraz centre at Bobo-Dioulasso was carried out in 1959–60; the tests used were Kolmer, VDRL and Kline in parallel, and a complement-fixation test with a Reiter antigen prepared by ultrasonic wave treatment. Of the total sera 662 were frankly positive and 257 doubtful; of these 662, 102 were from early cases [of treponematosis] 46 from late and 331 from latent cases. From these it is clear that in early cases antiprotein (Reiter) antibodies usually appear at the same time as reagin, sometimes later but never before; in late cases the antiprotein antibodies usually disappear before reagin, and in latent cases they do much the same, and they never remain positive when classical tests are negative; also in 2% of cases the Reiter test agrees with Kolmer (positive) when flocculation tests are negative. Of 257 doubtful results Reiter tests were positive alone in 16·5% and these are therefore considered false positives; in 13·5% there was complete agreement and in 70% partial disagreement.

Of 662 prenatal sera 31 were frankly positive and 25 doubtful; of the 25, Reiter alone was positive in 5, all were positive in 4 and in 16 there was disagreement; of the 25 in 4 Reiter agreed and in 5 it disagreed. In treated cases the Reiter test generally reverts to negative first, it is never positive alone and in some cases where it was negative before treatment it became positive later.

It seems therefore that the Reiter test is of no great value when other serum tests are doubtful, and it cannot replace the TPI. *T. E. Osmond*

Covert, S. C., Kent, J. F. & Stevens, R. W. **Fluorescent Treponemal Antibody Test using the Reiter Treponeme.** *Proc. Soc. Exper. Biol. & Med.* 1961, Apr., v. 106, No. 4, 729–31.

Because antigen made from virulent spirochaetes in the FTA test [this *Bulletin*, 1960, v. 35, 994] is very expensive that made from Reiter spirochaetes was used in carrying out fluorescent treponemal antibody tests (RFTA). Spirochaetes were cultured in the usual way and suspended in sodium hypochlorite (NaOCl, 0·002% in normal saline); they were washed, centrifuged and finally resuspended in saline to make a count of 10–15 per 43× microscopic field; virulent Nichols spirochaetes were used in a suspension 3–4 times as strong. For the test proper 0·01 ml. of antigen was placed in a circle on a slide and air-dried; slides were steeped in acetone and dried; 0·03 ml. of heated test serum diluted 1 in 200 in buffered saline was added to the antigen on the slide which was rotated at 100 r.p.m. for 30 minutes and dried with filter paper; 0·03 ml. of fluorescein-labelled horse anti-human-globulin diluted 1 in 30 in buffered saline containing 2% Tween 80 was then added, the slide was rotated 30 minutes at 100 r.p.m. in the incubator mounted with a cover slip and examined by special illumination.

When the results of the 2 types of test were compared on 51 sera from persons with syphilis they were much the same but the RFTA titre was usually higher than the FTA. Both tests were negative with sera from 10 healthy persons; of 38 sera from persons with other diseases who had given biological false positive reactions with classical tests 1 reacted with RFTA but none with FTA. *T. E. Osmond*

Dickman, A. **Experience with the *Treponema pallidum* Immobilization Test in a Private Laboratory.** *Pub. Health Lab.* 1961, Mar., v. 19, No. 2, 23–8.

There appeared to be only one private laboratory carrying out the TPI test in the world according to a survey in 1954, mainly because the procedure is expensive and complicated. After some preliminary remarks on the history of this test and on other treponemal tests Dickman reports his own experience. He calculates that the cost is not less than 17 to 25 dollars per specimen and he now carries out about 250 tests per year. His technique is on usual lines; complement is lyophilized guineapigs and rabbits are fed on antibiotic-free food the basal or survival medium keeps well at −20°C suspended tissue [presumably macerated syphilitic testes] is agitated by a magnetic stirrer in the incubator and for microscopy the high dry objective, a dry dark-field condenser and Ortholux illuminator are employed. Other materials recommended are *black* rubber stoppers and plastic tubes with polythene caps; whole clotted blood is the best type of specimen and penicillinase is used in all tests.

Results obtained on "problem" specimens showed that of 796 positive with VDRL 361 were confirmed by TPI and of 330 negative 258 were confirmed; corresponding figures for the Kolmer CF Test were 724 and

339 and 260 and 225; thus 54·6% of VDRL tests and 53·2% of Kolmer tests were probably biological false positives. It is suggested that negative VDRL and Kolmer tests where TPI is positive may be the result of time and treatment. It is of interest to note that the author gives figures to show that biological false positive reactions do *not* tend to be of low titre.

T. E. Osmond

KITNAR, E., VLASÁK, Z. & WEBERSCHINKE, J. **Demonstration of Specific Antibodies against *Treponema pallidum* in an Animal Test.** *J. Hyg., Epidemiol., Microbiol. & Immunol.* Prague. 1961, v. 5, No. 2, 241–7.

These authors tried to elaborate a method to demonstrate specific antibodies against *Treponema pallidum* by an animal test. A suspension of Nichols spirochaetes was made in the usual way from syphilitic testes and adjusted to contain 80–150 (average 118) organisms per field; guineapigs were used as experimental animals and their complement titres were determined; they were given ample food and water and kept cool; test sera were kept at 4°C. For the test proper a mixture of spirochaete suspension and test serum in the ratio of 4 ml. to 2 ml. was injected intraperitoneally into a guineapig after incubation at 37°C. for 30 minutes; specimens from the peritoneal cavity were withdrawn after 1, 2, 3, 4 and 7 hours and examined for motility, optimal times being 1 and 4 hours.

53 sera, 36 negative and 17 syphilitic, were tested; with the negative sera after 1 hour, 78 motile organisms were counted, varying from 59 to 95, and after 4 hours non-motile organisms never exceeded 2%; with 17 syphilitic sera the number of organisms was always less than 10% of those injected after 1 hour and after 4 hours never more than 3 could be seen.

In order to determine the type of antibody involved, living spirochaetes were incubated with syphilitic serum and complement for 16 hours at 35°C. but the organisms did not disappear; killed spirochaetes incubated with positive sera showed no appreciable agglutination.

It is concluded therefore that syphilitic antibodies contain immobilisins and adhesins but not bacteriolysins or agglutinins.

T. E. Osmond

GREAVES, A. B. **Further Observations on Penicillinase.** *J. Amer. Med. Ass.* 1961, June 17, v. 176, No. 11, 951–3.

In order to investigate the action of penicillinase this author treated two series, each of 10 patients with early syphilis, with penicillin alone and with the same plus penicillinase; the former received 600,000 units of PAM and the latter the same but with 800,000 units of penicillinase 2 hours previously. Dark-ground examinations were carried out hourly and no definite differences in the disappearance time of spirochaetes was noted; the mean for the first series was 14·75 and for the second 17·6 hours. On the other hand penicillin was detectable after 8 hours in all of 4 of the penicillin subjects studied but in none (with one slight exception) of the penicillin plus penicillinase group of 10; moreover it was found that chancres in this latter group healed just as rapidly as in the penicillin group. In the case of gonorrhoea it was found that penicillin plus penicillinase failed to cure the disease and that patients given penicillinase for side reactions needed other antibiotics for cure.

[Blood levels of penicillin are given as units per millimetre, presumably per millilitre is meant; surely the plural of the organism of syphilis is treponemata or treponemes, not treponemas.]

[See also this *Bulletin*, 1956, v. 31, 1034.].

T. E. Osmond

CHEW PIN KEE. **Gonorrhoea in School Children.** *Singapore Med. J.* 1961, Mar., v. 2, No. 1, 30–33, 1 fig.

During the first 11 months of 1960, 25 cases of suspected gonorrhoea were found among schoolchildren attending the School Health Clinic, Singapore. The average daily attendance at this Clinic is about 350–400.

The 25 patients included 15 boys and 10 girls; smears were taken from the urethra in 13, vagina in 9 and conjunctiva in 3; only 6 (all boys) were positive for gonococci. Short histories of these are included; none appear to have been venereal infections, lavatory seats, swimming pool, bathroom etc. being claimed as the source; ages varied from 9 to 16 years.

T. E. Osmond

BULL. WORLD HEALTH ORGANIZATION. Geneva. 1961, v. 24, No. 3, 293–384, illustrated. [Numerous refs.] **Gonorrhoea. Treatment Problems; Gonococcal Resistance to Antibiotics; Epidemiology.**

The 10 articles by different authors considered here deal with the various problems concerning the control of gonorrhoea.

In the introduction (pp. 293–296) it is pointed out that the incidence of gonorrhoea has increased in 15 out of 22 countries surveyed, that the group aged 15–19 years shows a rising attack rate and that repeat infections are becoming commoner; control therefore is mainly an epidemiological problem. T. GUTHE (pp. 297–306) stresses the points that the short incubation period makes tracing of contacts difficult, that females constitute a reservoir of infection, that, though complications in general have diminished, salpingitis and consequent sterility are not uncommon and that the gonococcus seems to be becoming more and more resistant to penicillin; improved sero-diagnostic methods and possible immunization are 2 aspects which demand study. He gives 4 figures showing the incidence of gonorrhoea and early syphilis in various countries from 1948 to 1959 and a table showing the sensitivity of the gonoccocus to penicillin as assessed by various authors.

R. R. WILLCOX (pp. 307–319) considers treatment problems; reasons for failure include misdiagnosis, re-infection which cannot be distinguished from relapse, persistence of infection due to factors such as inadequate dosage, failure of the antibiotic to reach the organisms,

destruction of penicillin by penicillinase and defective host defence. Sulphonamides seem to have recovered their gonococcocidal properties, but decreasing sensitivity of the organisms to penicillin seems to be clear; assessment of this is rendered difficult by the variety of methods employed; these should be standardized. The arguments for and against rapidly acting and slowly acting preparations are discussed, particularly with reference to concomitantly acquired syphilis. Tables showing sensitivity to penicillin, sulphonamides, streptomycin and other antibiotics are included.

C. M. Carpenter (pp. 321–326) gives a critical review of the literature on penicillin resistance; he regards absolute resistance as unlikely, usually it is only partial. To investigate the problem it is necessary to measure penicillin blood levels, to exclude re-infection, to confirm diagnosis by culture, to eliminate host factors, to estimate the *in vitro* resistance of the strains involved and to rule out the effect of penicillinase. 2 problems to be solved are the possible PPLO inversion forms of the gonococcus and the presence of a concomitant non-gonococcal urethritis in the patient.

J. D. Thayer, Frances W. Field, Miriam I. Perry, J. E. Martin, Jr., and W. Garson (pp. 327–331) discuss surveillance studies of *Neisseria gonorrhoea* and sensitivity to penicillin and 9 other antibiotics; in two tables are shown penicillin susceptibility of 368 strains, in 70·1% of which it was to 0·05 units per ml. or less, and of 85 cultures; all the latter except 2 were susceptible to 0·30 units per ml. or less. The *in vitro* average susceptibility of 9 antibiotics was as follows (units per ml.), kanamycin 8·30, leucomycin 0·55, chloramphenicol 0·44, dextrosulphenidol 0·28, chlortetracycline 0·26, oxytetracycline 0·19, tetracycline 0·18, demethylchlortetracycline 0·09 and Synnematin B 0·98. Strains clinically resistant are not always found to be so *in vitro*, host factors vary and phagocytosed organisms may be "protected". A standardized system of assaying resistance is not only desirable but essential.

Alice Reyn, and M. W. Bentzon (pp. 333–342) discuss the possible effects of antibiotic treatment on the sensitivity and growth requirements of *Neisseria gonorrhoeae*. They refer to their previous report where they found that 25% of strains in 1959 required 20 times as much penicillin to kill as in 1944; they also encountered strains completely resistant to streptomycin but none to tetracycline; atypical strains were also met. They tested sensitivity by the tablet and dilution methods. 20 strains subcultured frequently retained their reduced sensitivity levels. Comparing strains in 1957 and 1958, they found that sensitive strains seemed to be killed off and resistant strains therefore increased.

Sensitivity to penicillin and to streptomycin was roughly parallel. After 1957 certain strains (up to 40%) showed atypical features such as poor growth, inability to ferment glucose, and the production of an anti-complementary antigen; however, it was found that ox-heart medium was better than ox muscle, and human placenta better than either, and these strains seem more sensitive to penicillin than typical strains.

P. Durel, Véra Roiron and Louise Delouche (pp. 343–348) discuss streptomycin which is routine for treatment in France; however, 23·2% of strains required at least 1,000 mgm./ml. to kill them whereas most of these were relatively sensitive to penicillin and all sensitive to tetracycline and spiramycin; in addition, sulphathiazole seems to have regained its efficacy. Bacteriological evidence supports the clinical evidence and no doubt streptomycin will have to be replaced for routine use.

W. E. Deacon (pp. 349–355) describes his researches into the question of identifying gonococci by fluorescent antibody (FA) methods, taking his cue from the behaviour of other organisms such as salmonellae, shigellae, and escherichiae, and the presence of a capsular antigen K, which is destroyed at 120°C. but not at 100°C. The gonococcus with K antigen is coloured brilliantly when treated with fluorescein-labelled antiserum (this is well shown in 6 figures); this should help in the diagnosis of gonorrhoea in the female. In addition FA may serve to distinguish various strains from one another.

R. R. Willcox (pp. 357–366) writes of the epidemiological aspects of gonorrhoea and points out not only that penicillin has not solved the problem but that, of 22 countries, in 15 the disease has increased and in only 4 has there been a sustained fall. Reasons suggested include general factors (lack of public interest), social ("repeaters" and immigrants and teenagers), reservoirs of infection which cannot be located, and diagnostic and treatment (increased resistance to penicillin) difficulties.

H. Gisslén, L. Hellgren and V. Starck (pp. 367–371) write of the incidence, age distribution and complications of gonorrhoea in Sweden; the disease has been increasing, especially in age-groups 15–19 and 20–24, though in these latter the increase is not likely to be maintained. Complications too have increased, especially salpingitis, but this increase may be explained in part by the fact that patients from general hospitals as well as from venereological divisions have been included. Figures and rates are given in 3 tables and 2 figures.

Finally A. J. King and C. S. Nicol (pp. 373–384) present a long article on the problem of gonorrhoea in England and Wales. The disease has increased in recent years after the marked post-war fall; the explanation lies partly in immigration, increased promiscuity of young people, undiscovered reservoirs of infection, difficulty of diagnosis in the female and increased penicillin resistance. The most important preventive measures are free and confidential treatment, health education, pre- and post-natal examinations, the fight against charlatans and a stricter surveillance of prostitutes. Numerous statistics are set out in 6 tables. *T. E. Osmond*

Medical Res. Council. **Resistance of Gonococci to Penicillin. Interim Report by a Medical Research Council Working Party appointed to examine the Resistance of Gonococci to Penicillin** [Fairbrother, R. W., Chairman]. *Lancet* 1961, July 29, 226–30. [23 refs.]

This working party investigated the sensitivity to penicillin of 1,984 strains of gonococci in 9 different centres in the United Kingdom during the period April 1959 to March 1960. Two groups were concerned: (1) those from patients before treatment, and (2) those from patients who were apparently "treatment failures".

Sensitivity was assessed by either a tube dilution technique or by plating. Of the 1,984 strains 262 (13·2%) required a concentration of 0·125–1·0 unit per ml. for inhibition; resistance was most noticeable in Sheffield and Wakefield and lowest in Liverpool and Edinburgh; in Manchester and Southampton resistance was high at first but declined later.

As regards strains from "treatment failures", 73 out of 137 were very sensitive *in vitro* being inhibited by 0·06 unit per ml. (some of these may have been reinfections); in 38 cases where reinfection could be ruled out 32 required 0·125–1·0 unit per ml. for inhibition; in many of these 1·2 mega units of PAM had failed. The working party considered that the present position "gives cause for concern but not alarm"; failure may be due to (1) reinfection, (2) low blood concentration of penicillin, or (3) decreased sensitivity of some strains of gonococci. Surveys should be continued in many different places by random sampling; considerably increased dosage of penicillin is recommended; streptomycin is not a satisfactory long-term alternative and it may be that sulphonamides would provide only a short-term solution of the problem. *T. E. Osmond*

Svihus, R. H., Lucero, E. M., Mikolajczyk, R. J. & Carter, E. E. **Gonorrhea-Like Syndrome caused by Penicillin-Resistant *Mimeae*.** *J. Amer. Med. Ass.* 1961, July 15, v. 177, No. 2, 121–4. [18 refs.]

So-called penicillin-resistant gonorrhoea has been the cause of much discussion. These authors investigated 42 U.S. service patients at Naples suffering from urethritis simulating gonorrhoea, by means of smears and cultures. Those apparently suffering from gonorrhoea were given 1,200,000 units of benzathine penicillin G plus 600,000 units of procaine penicillin G; others were treated with various other antibiotics.

Of the 42 patients, 37 had a thick purulent and 5 a thin watery discharge. Of the 37, 34 showed gonococcus-like colonies which proved to be gonococci in 11, *Neisseria flava* in 1, *Mima polymorpha* var. *oxidans* in 10, *M. polymorpha* in 6 and other Mimeae in 6; the other 3 grew *Escherichia coli communior* in 1, *Flavobacterium* in 1 and unidentified in 1. The 5 others grew *Staphylococcus epidermidis* 3, diphtheroids 1, and *Streptococcus viridans* 1. (The Mimeae include *M. polymorpha, M. polymorpha* var. *oxidans, Herellea vaginicola* and *Colloides anoxydana* [this *Bulletin*, 1943, v. 18, 957].)

All 12 patients with *Neisseria* responded to penicillin, as did 4 of those with Mimeae; the other 18 whose organisms were penicillin resistant responded to one or other broad-spectrum antibiotic.

Details of the various laboratory procedures are given in the text. Further studies seem indicated to discover how widespread is infection with the Mimeae.

T. E. Osmond

Jacobs, A. G. **A Case of Reiter's Syndrome in Childhood.** [Memoranda.] *Brit. Med. J.* 1961, July 15, 155, 1 fig.

After pointing out that this condition is comparatively rare in children this author describes the case of a boy aged 9 who complained of pain on micturition; he had recently had abdominal pain and diarrhoea and previously balanitis and acute nephritis. On examination his temperature was 99°F. and his urine contained a little protein, many pus cells and a few epithelial and red cells. He was treated with sulphamethizole, potassium citrate and penicillin; next day his temperature was 100°F. Dysuria persisted and his right knee was painful; next day the knee was more painful, temperature 101°F. and there was discharge from both eyes.

Investigations showed Hb 84%, sedimentation rate 56 mm./hour, white cell count 18,400 per cmm. (stab cells 2%, polymorphs 78%, lymphocytes 14%, monocytes 6%); no antibodies to the *Shigella* group were detected nor growth of any virus.

After 2 days signs and symptoms improved, sedimentation rate was 62 mm./hour and polymorphonuclears were 63%. By the 10th day the patient was much better but the conjunctivitis relapsed on the 21st and 23rd days, but cleared with hydrocortisone drops; sedimentation rate finally fell to 7 mm./hour. Recovery was complete in 6 weeks. *T. E. Osmond*

Röckl, H., Borelli, S. & Hardieck, L. Behandlungsergebnisse mit dem Imidazolderivat Clont bei der Trichomoniasis des Menschen. **[Treatment of Trichomoniasis with Clont, an Imidazole Derivative]** *Deut. med. Woch.* 1961, June 9, v. 86, No. 23, 1130–32, 1163.

Several reports have been published on the value in the treatment of trichomoniasis of 1-hydroxyethyl-2-methyl-5-nitroimidazole which is marketed under the name Clont by Bayer A.G. Leverkusen, Rhine, and in France and Canada under the name Flagyl by the firm of Poulenc.

In this paper are reported the results of treatment of *Trichomonas* infection of 98 women with leucorrhoea and 20 men with urethritis and prostatitis. The drug may may be given orally and the usual dosage for women was 250 mgm. given twice in 24 hours for 5 days, and for men the same treatment for a period of 3–5 days. It was not possible to carry out an adequate follow-up examination of all these patients but among 16 females who could only be examined once on the day after the end of treatment 11 gave a negative result, and among 52 women examined between 1 week and 4 months after treatment all but 3 were negative. All the 20 males appear to have been cured after only 1 course of treatment. It is considered that no concomitant local application of the drug is necessary. From the experience here recorded and also the observation that there were no important side-effects the conclusion is that Clont is the most effective agent against human trichomoniasis that as yet exists. *M. E. Delafield*

Gadgil, R. K., Mansukhani, S. H. & Irani, S. B. **Laboratory Control in Therapy of Vaginitis.** *J. Indian Med. Ass.* 1961, June 1, v. 36, No. 11, 513–15.

These authors treated 100 patients suffering from vaginitis by two methods; patients included 45 with

Candida albicans, 36 with *Trichomonas vaginalis* and 19 in whom no organisms could be incriminated. The first 50 were treated with di-iodohydroxyquinoline, phenyl mercury nitrate and neomycin in a base of sodium lauryl sulphate; of 21 with *T. vaginalis* 14 were "cured" [? clinically], 11 were cured but "culture positive" and in 7 response was poor; in 17 with *Candida* infection the corresponding figures were 12, 7 and 5 and in 12 "others" they were 5, *nil* and 7.

The second 50 received the same 3 drugs in association with dioctyl sodium sulphosuccinate plus amylocaine an amethocaine; of 15 patients with *T. vaginalis* 13 wer cured, 3 cured [clinically] but culture was positive an 2 showed no response; the corresponding figures for 2 with *Candida* were 19, 14 and 19, and for 7 "others 4, *nil* and 3.

Thus it appears that if test of cure includes cultur few patients were really cured [fortunately Flagyl ha recently been shown to cure a large proportion of case this *Bulletin*, 1961, v. 36, 145, 146]. *T. E. Osmond*

GENERAL INFECTIVE DISEASES

The order in this section is:—**General; Hospital and other Cross Infections; Coccal Infections; Bacterial Infections; Virus Infections.**

HOSPITAL AND OTHER CROSS INFECTIONS

COPE, Anna, SHOOTER, R. A., GREEN, Susan M. & NOBLE, W. C. **Nasal Carriage of *Staph. aureus* by Newborn Babies.** *Brit. Med. J.* 1961, Aug. 5, 329–30.

In extension of previous studies reported from St. Bartholomew's Hospital, London [this *Bulletin*, 1960, v. 35, 783], the authors have observed the effect of applying neomycin cream once daily to the noses of newborn babies, whose skin was also dusted at frequent intervals with hexachlorophane powder. The nurses used hexachlorophane soap. The babies' noses were examined bacteriologically each day.

Of 221 babies only 38 (17%) were found to carry *Staph. aureus* in the nose at any time and 20 of them carried on 1 day only. 2·7% of the babies developed mild staphylococcal lesions of skin or eye and a further 1·8% had lesions that were not swabbed. The staphylococcal sepsis rate was, however, 10·5% among the 38 babies who became carriers. *R. E. O. Williams*

HARE, R. & COOKE, E. M. **Self-Contamination of Patients with Staphylococcal Infections.** *Brit. Med. J.* 1961, Aug. 5, 333–6.

Studies were made of the nose and various skin areas of 10 patients with *Staph. aureus* wound infection and of 11 with other staphylococcal disease. Of the 10 with wound infections, 2—those with the most severe infections—showed widespread contamination but 4 yielded very few staphylococci from any site other than the wound. Patients with infected eczema and the like had widespread contamination with staphylococci; those with localized infections had much less. The patients who are active dispersers contaminate their environment very extensively and it seems likely that their staphylococci can be contained only by isolation measures.

R. E. O. Williams

MACRÌ, N. Sulla incidenza di portatori di stafilococc nei ricoverati e nel personale di assistenza dell'ospe dale militare di Messina. **[Incidence of Staphylo coccal Carriers in Patients and Staff of th Military Hospital in Messina]** *Nuovi Ann d'Igiene e Microbiol.* 1961, Jan.–Feb., v. 12, No. 1 37–57. [101 refs.]

The English summary appended to the paper is a follows:—

"The incidence of the staphylococcal nasal carrier has been studied in the patients and the staff of th surgical departments of the military Hospital in Messina.

"22 per cent of pathogenic strains have been isolate from the patients' noses; in the staff the percentage ha been slightly lower. The percentage of strains resistan to antibiotics is very near to the isolated strains outsid Hospital environment.

"The environment and the nosological features of th military Hospitals are not propitious for staphylococca diffusion."

HARDING, J. W. & THOMPSON, R. E. M. **Results o Treatment of Hospital Staphylococcal Infection with Methicillin.** *Postgraduate Med. J.* 196 Aug., v. 37, No. 430, 485–9, 1 fig.

"1. Eleven cases treated with methicillin are recorde and in all cases staphylococcal infection was eliminate

"2. No case showed any evidence of toxicity due t the drug, although acquired hypersensitivity wa suspected in one case."

CRAINZ, F., LOMBARDO, G. & ROMAGNOLI, G. Un ep sodio epidemico di salmonellosi nei lattanti. [**Salmonellosis Outbreak in Young Infants]** *Ri Italiana d'Igiene.* 1960, Nov.–Dec., v. 20, Nos. 11 12, 487–91. [12 refs.] English summary (4 lines).

This outbreak occurred in maternity wards at Nova Hospital. The first case set in on December 1st, wi

severe diarrhaea, in a baby born on November 27th: the parents took the baby away from hospital on December 10th and he died at home the next day. 14 other infants in these wards were taken ill during December 16–20: the illness was mild in 8 and they soon recovered, there were more serious manifestations in 3 others who also recovered, and the remaining 3 died. Stool culture produced *Salmonella enteritidis* but serological investigation proved negative against *Salmonella enteritidis, Salm. typhi, Salm. paratyphi* [unspecified] and *Salm. cholerae suis.*

The patients were treated with tetracycline followed by chloramphenicol, and preventive measures included isolation and terminal disinfection. Bacteriological investigations were continued till the next June and many of the children who recovered went on excreting the salmonellae for several months. None of the infants born before November 26th was found infected, but the mother of the first patient, who had come into hospital on that date, was found infected when her stools were examined 2 months later and she is assumed to have been responsible for bringing *Salm. enteritidis* into the wards and thus causing the outbreak. At some time or other the organism was recovered from 5 of the mothers and from 3 of the relatives, but investigation of the hospital staff at all levels was negative. Bacteriological results are graphically set out. *J. Cauchi*

COCCAL INFECTIONS

The order in this section is:—***Staphylococcus, Streptococcus, Neisseria, Others.***

HOFFMAN, E. **Empyema in Childhood.** *Thorax.* 1961, June, v. 16, No. 2, 128–37, 9 figs.

This paper deals with 125 patients aged from 2 months to 12 years who were admitted to the thoracic surgical unit, at Shotley Bridge, Newcastle-upon-Tyne, between 1941 and 1958. 112 were classified as having acute empyema, the remaining 13 were in the chronic category. A graph is produced showing the number of patients admitted with empyema annually plotted against the date of introduction of the various antimicrobial drugs. The numbers admitted are divided into two: children aged 2–12 years, and children aged 0–2 years. In the former group there were 22 cases in 1944, and this fell steadily until 1956 onwards when there was only one. There were 6 patients under 2 years old in 1944, rising slightly, followed by a fairly rapid fall until in 1954 there were none. Since then, however, the number has risen again to 5. A second graph shows the relative incidence of pneumococcal and staphylococcal empyema up to the age of 12 years. The graph for pneumococcal infection is practically the same as that for children aged 2–12, whereas the graph for staphylococcal empyema follows that shown in the previous graph for children aged 0–2 years, *i.e.*, pneumococcal empyema is becoming rare whereas staphylococcal empyema, mainly in children below the age of 2, is almost as common as it was 15 years previously. A review of the whole period shows that the pneumococcus was the commonest offender, accounting for 55 cases. *Staphylococcus pyogenes* was responsible for 28, and haemolytic streptococci for 12. There was one case caused by *Haemophilus influenzae* and one by *Klebsiella pneumoniae.*

In 22 patients the laboratory reported the pus to be sterile, mainly because of previous antibiotic therapy. Of the 28 patients who had staphylococcal infection, 17 were under 2 years of age, and 11 were babies under 1 year of age. Of the babies under 1 year of age, 11 had a staphylococcal infection, and 2 a streptococcal infection; there were no cases of a pneumococcal infection. A table is also given of reports on primary staphylococcal pneumonia, and its complications in infants under 2 years of age as detailed by 4 authors [this *Bulletin*, 1956, v. 31 243, 887; 1958, v. 33, 819]; these all refer to patients who had the disease from 1952 onwards. The figures quoted by these authors show that a pneumothorax occurred in 25% to 33%, and a pneumatocele in 16–66%. The complications in the present series included suppurative pericarditis in 1 patient, septicaemia in 6, meningitis and cerebral abscess in 2, and bronchiectasis in 4. There were 5 deaths.

A table is given showing the sensitivity to antibiotics in 37 patients according to the year. Staphylococcal infections became steadily more frequent, and increasingly resistant to penicillin and to streptomycin. As regards mechanical methods of treatment, rib resection very common in 1940–50 has now become obsolete, but intercostal drainage, after sinking rapidly by 1954 has become much commoner in the last few years. Bronchograms were carried out in 56 of the patients, and gross bronchiectasis was found in 7, of whom 3 had chronic empyemata. In 3 patients the bronchiectasis was the cause of the empyema. Bronchograms show that the least lung damage occurred in the pneumococcal and staphylococcal cases; the streptococcus was responsible for a somewhat greater degree of lung damage, and finally chronic empyema caused the highest incidence of bronchial complications.

[This is a very interesting paper, the only criticism being that in some observations clear differentiation is not made between patients seen in the pre- and post-antibiotic era.] *F. H. Young*

ESRACHOWITZ, Sheila R. **Pyogenic Meningitis—a Study of 303 Cases.** *South African Med. J.* 1961, Feb. 11, v. 35, No. 6, 101–4. [17 refs.]

This is a useful report on the results of treatment of 303 cases of pyogenic meningitis of all ages admitted to the City Hospital for Infectious Diseases, Cape Town, during the 3 years 1955–57. There were 198 proved meningococcal cases, 61 cases of purulent meningitis (presumed meningococcal), 20 of pneumococcal, and 24 of *Haemophilus influenzae* meningitis. The survival rates were 94% in meningococcal, 87% in purulent, 85% in pneumococcal, and 79% in *H. influenzae* meningitis. A considerable improvement on these figures was noted when deaths within 24 hours of admission were excluded.

Obviously, therapy in pyogenic meningitis is now highly efficient, and the main source of improvement in results

is to be sought in earlier diagnosis and treatment. Thus the average number of days of illness before admission for pneumococcal meningitis was 7 overall but 13·5 in those who died, and for *H. influenzae* meningitis 11 overall, but 22 in those who died. Awareness of the early signs and earlier resort to lumbar puncture, hold out most hope for further improvement in results.

H. Stanley Banks

MURPHY, E. F. **Invasive Staphylococcal Disease.** *Irish J. Med. Sci.* 1961, June, 6th Ser., No. 426, 257–67.

The author reviews a series of 87 cases of staphylococcal septicaemia, a condition which he prefers to call "invasive staphylococcal disease". The patients were of all ages from a few months to over 60 years. The disease was always confirmed bacteriologically, but the author stresses the importance of urgent early treatment based on clinical diagnosis. He describes the symptomatology under the headings of gastro-intestinal, pneumonic, neurological, cardiac, renal, skin, bone and adrenal, and he discusses the outlines of treatment. There were 18 deaths, 8 occurring within 24 hours of admission. [This is a useful, concise, clinical article which cannot be adequately abstracted.] *A. B. Christie*

STREITFELD, M. M. & SASLAW, M. S. **Correlation of Population Age with Recovery Rates of β-Hemolytic Streptococci and Serological Responses: Relationship to Rheumatic Fever.** *J. Infect. Dis.* 1961, May–June, v. 108, No. 3, 270–77, 1 fig. [18 refs.]

"Cultures taken from healthy children's throats yielded more β-hemolytic streptococci than did cultures from healthy adults' throats. In children 6 to 9 years of age, the streptococcal recovery rate was highest (β-hemolytic streptococci of all groups, 25.9%; group A only, 14.4%). Children 12 to 15 years of age had lower rates (18.6 and 7.9%, respectively). Adults had the lowest rates (Miami residents, 9.0 and 1.9%; airline employees, 12.0 and 2.4%). The difference in rates was due mainly to the large numbers of group A organisms recovered from children.

"Average antistreptolysin O titers tended to be higher in children than in adults, both for total streptococcal isolate and for most individual groups of β-hemolytic streptococci. However, those adults who harbored β-hemolytic streptococci manifested ASO responses in their serums to the same degree as children.

"The higher streptococcal recovery rates and higher average antistreptolysin O titers in children than in adults parallel the higher incidence rate of acute rheumatic fever in children reported by others."

POPOV, N. **Serological Investigations into Sequelae of Scarlet Fever with particular reference to Rheumatic Fever.** *Arch. Dis. in Childhood.* 1961, Feb., v. 36, No. 185, 77–81, 2 figs. [14 refs.]

Nine different laboratory tests were carried out in a series of 31 children in the post-scarlatinal period for 1 to 3 years. In the first 6 months 27 (87%) had complications or intercurrent disease—tonsillitis in 20, arthralgia in 8, myocardial weakness in 3, lymphadenitis in 5, otitis media in 3, and allergic manifestations in 5; none had rheumatic fever. The tests for E.S.R., C-reactive protein, serum protein fractions, and fibrinogen level gave results related to the focal inflammations present. The indices of a rheumatic attack (severe disproportion of serum proteins, hyperfibrinogenaemia, and high antistreptolysin titre) were not found. There was no ground for considering that the post-scarlet-fever period was immunobiologically identical with the acute or pre-acute phase of rheumatic fever. *H. Stanley Banks*

BACTERIAL INFECTIONS

The order in this section is:—***General, Corynebacterium, Haemophilus, Enteric bacteria, Brucella Pasteurella, Bacillus, Anaerobes, Spirochaetes, Others.***

KUBINYI, L. Ivóvíz által terjesztett járványok Magyarországon az utolsó 10 évben. [**Epidemics spread by Drinking Water during the Last Ten Years in Hungary**] *Országos Közegészségügyi Intézet Müködése, 1959.* Budapest. 1961, 262–8. [10 refs.]

The English summary appended to the paper is as follows:—

"The author has collected the data referring to water borne epidemics during the last ten years in Hungary, and drawn conclusions regarding the frequency of technical causes leading to the contamination of drinking water and the rôle of water in the spread of some infectious enteric diseases, 38 epidemics affected a total of 49,233 persons; enteritis was responsible for 92.5 per cent dysentery for 6.8 per cent, typhoid for 0.4 per cent, and hepatitis for 0.3 per cent. 78.9 per cent of the epidemics under review and 98.8 per cent of the cases involved were spread by water-conduit. Wells still play an important rôle in the spread of typhoid fever at villages. Contamination of running water was due to lack of protective areas and the propinquity of sewers in 66 per cent, to inflow of industrial water or mine water in 20 per cent to defects of watermains and consequent access of sewage in 14 per cent. The number of epidemics has shown an increase in recent years. The technical state of the waterworks is unsatisfactory. Hygienic prescriptions are often disregarded in the construction of new buildings. Intensified control of the waterworks appears to be indispensable."

SOOD, S. C. & TANEJA, P. N. **Typhoid Fever. Clinical Picture and Diagnosis.** *Indian J. Child Health* 1961, Feb., v. 10, No. 2, 69–76. [19 refs.]

This useful article describes the clinical condition and diagnostic features of typhoid fever in 95 children in

whom the diagnosis was confirmed by either blood culture or clot culture of *Salmonella typhi*. It is unusual for clinical descriptions of typhoid fever in children to be based on such relatively ample material. Noteworthy features that emerged are: (1) the very variable clinical patterns including the frequency of delirium and restlessness, absence (in coloured children) of characteristic rose spots, absence of abdominal distension, almost equal distribution of diarrhoea and constipation (each about 17%), chest signs or cough in 44%, palpable spleen in 40%; (2) the small value of total and differential blood counts, although slight lymphocytosis was usual in children over 8 years of age; (3) the suggestive value only of a Widal test; (4) the surest method of diagnosis being *both* blood culture and clot culture, either of which, singly, not infrequently failed. *H. Stanley Banks*

BAYLET, R. J., ARMENGAUD, M. & KERREST, J., with the technical collaboration of N. PINHEDE. Isolement des Salmonelles à partir de ganglions mésentériques humains. **[Isolation of Salmonellae from Mesenteric Lymph Nodes in Man]** *Bull. Soc. Path. Exot.* 1960, Sept.–Oct., v. 53, No. 5, 779–81.

In a series of 20, apparently unselected, post-mortem examinations salmonella organisms were isolated from the mesenteric lymph nodes from 5 of the subjects. The report suggests that in no case was a salmonella infection suspected during life, the terminal illnesses recorded as the cause of death being chronic nephritis, infantile anaemia, syphilitic aortitis, measles with complications and malignant malaria. *Salmonella typhi* was isolated from the mesenteric glands of the subject with malaria, and this would seem to be an example of a double infection where the typhoid infection may well have been responsible for a malarial relapse which then masked the symptoms of the typhoid fever. In the other cases it is possible that the primary disease provided conditions which allowed the salmonella organisms to establish themselves in the bowel, which normally they may well not have done, and invade the mesenteric glands. Whether the salmonella infections were a contributory cause of death it is not possible to say, except for the typhoid case in which there seems to be little doubt.

The report of these findings comes from Dakar where salmonella infections are very common, the serotypes responsible are extremely varied and many of the types are not commonly seen in temperate countries.

H. J. Bensted

LEONESCU, M. & HORODNICEANU, Thea. Valoarea fagodiagnosticului în dizenterie. **[The Value of Phage Diagnosis in Dysentery]** *Microbiol., Parazitol., Epidemiol.* Bucharest. 1961, May–June, v. 6, No. 3, 271–7.

The English summary appended to the paper is as follows:—

"A study is carried out on the value of phage diagnosis in dysentery, using the anti-flexneri Sartorius D. phage.

"The method used was that of increase of the phage titre in the faeces, described by I. Schechter for typhoid fever.

"Reactions for the increase of the phage titre were performed in 154 cases, of which 84 dysentery patients and 70 patients suffering from other affections than dysentery served as controls.

"The conclusion is drawn that the method of increase of the phage titre in dysentery patients is insufficiently specific to constitute a method for the bacteriologic confirmation of dysentery.

"The reaction for the increase of the phage titre in the faeces is useful for indications of an epidemiologic order (the presence in the intestines of germs with a Shigella-like antigenic structure) particularly for *Sh. flexneri*."

RAUSS, K., BÁRÁSZ, Z. & KÉTYI, I. **Immunological Investigation of Patients suffering from Chronic Dysentery and of Chronic Carriers.** *J. Hyg., Epidemiol., Microbiol. & Immunol.* Prague. 1960, v. 4, No. 2, 142–50. [37 refs.]

Transitory healthy carriers of dysentery bacilli are healthy persons without any history of clinical dysentery or endoscopic evidence of any intestinal lesion, but from whom dysentery bacilli have been recovered on one occasion. The sera of such persons do not contain any protective antibodies.

Asymptomatic chronic carriers are those without any history of previous dysentery or clinical or endoscopic evidence of disease but who have been persistently excreting dysentery for a month or more; their sera frequently contain high-titre mouse-protecting antibodies. Those suffering from chronic dysentery show bacteriological, clinical and endoscopic evidence of infection and their sera also contain mouse-protecting antibodies. These two conditions are considered to be closely related and it is suggested that chronic dysentery may be but an allergic form of the asymptomatic chronic carrier state; and this latter condition finds its parallel in typhoid fever in the chronic typhoid carrier whose serum contains significant amounts of mouse-protecting antibodies against a typhoid challenge. *H. J. Bensted*

SPENCER, F. J. **Epidemic Shigellosis in a Rural Area.** *Pub. Health Rep.* Wash. 1961, Mar., v. 76, No. 3, 219–22, 3 figs.

This short paper gives brief accounts of 3 outbreaks of bacillary dysentery in Virgina with different epidemiological pictures. The first, due to infection with one of the Flexner organisms, affected 79 students out of a total of 1,502 in a women's college and was more or less explosive in nature. The infection was traced to the dining hall of the college where it appeared that the affected persons were served with meals by a waitress who was subsequently shown to be suffering from Flexner dysentery. The story is not quite complete but there is little doubt the infection was acquired in the students' dining hall and the outbreak may be said to be food-borne.

The other two outbreaks were small in size and both were due to Sonne's bacillus and infection spread by direct

contact. The origin of the first of these two was not traced, but the second is of interest as an illustration of the infectiveness of children with Sonne dysentery. The infection was introduced into the family by one of the children and spread to the other members of the family and thence to others. *H. J. Bensted*

SZMUNESS, W. Epidemiologie der Ruhr des Sonnei-Typs. [**Epidemiology of Sonne Dysentery**] *Ztschr. f.d.g. Hyg. u. ihre Grenzgebiete.* Berlin. 1961, June, v. 7, No. 6, 465–71, 3 figs. [11 refs.]

During the last few years reports on *Shigella sonnei* dysentery from many countries have recorded considerable increase in the frequency of this infection and also that it represents a greater proportion of the total of dysentery cases.

In this paper the author describes investigations of 511 Sonne dysentery cases which occurred in some large cities of Poland in the last few years. These cases were analysed from the clinical, epidemiological and microbiological viewpoints. In the present communication only the epidemiological findings are described, and they are compared with those obtained in 589 cases of *Shigella flexneri* dysentery which occurred in the same cities at the same time.

There is a careful statement of the comparative findings with figures and tables. Among the general conclusions are:—(1) on an average Sonne dysentery represents 30% of the total of dysentery but fluctuating considerably in different years in a range of 9·2% to 54%; (2) the summer and early autumn months are the times of highest incidence of the Sonne infection with relatively few cases in the other months. The Flexner dysentery curve of incidence is somewhat more uniform than this; (3) the age distribution of the 2 infections is different, *e.g.* at age up to 2 years Flexner is more frequent than Sonne dysentery but at 20–49 years this finding is reversed; (4) family infections are much fewer in Sonne than in Flexner dysentery; (5) no concentration of cases of Sonne dysentery in the poor districts was found; and (6) in Sonne dysentery patients cease to excrete the causative organism more quickly than do the Flexner patients and it was also found that isolation of the organisms from the faeces is less frequently achieved in Sonne than in Flexner dysentery. *M. E. Delafield*

MERZBACH, D. & FALK, W. [***Escherichia coli* 0126: B16 in Rambam Hospital in the Years 1956–1960**] *Harefuah.* Jerusalem. 1961, Aug. 1, v. 61, No. 3, [in Hebrew 92–8, 2 charts. (30 refs.) English summary 100].

The English summary appended to the paper is as follows:—

"*Escherichia coli* O126:B16 serotype (Taylor J. and Charter R. E.) was isolated in 153 cases hospitalized in the two pediatric departments of this hospital.

"In one small outbreak of 16 cases in 1956/7 half of the cases showed parallelism between the appearance of gastroenteritis and the serotype findings. In the majority of the 137 other cases in the years 1956—1960 nosocomial infection can be excluded.

"140 cases (91%) included infants of up to 2 years; 117 of them (76% of all cases) showed clinical symptoms of gastroenteritis—no other etiological agent having been found. In 74 cases (48%) the symptoms were of severe or moderate nature. In 94 cases (61%) the serotype was isolated in rich growth from stool specimens taken within 3 days of admission.

"It appears that in the majority of the above cases this serotype might be the etiological agent of the gastroenteritis."

TASSELLI, E. L'endemia brucellare in provincia di Foggia. [**Epidemiology of Brucellosis in the Province of Foggia**] *Ann. d. San. Pubblica.* Rome. 1961, Mar.–Apr., v. 22, No. 2, 255–301, 4 graphs & 1 map on folding pl. [22 refs.] English summary.

The author is veterinary officer in the province of Foggia, in the region of Apulia, Italy. He records the annual notifications of human brucellosis in Italy during 1930–59 and the corresponding morbidity rates calculated per 10,000 inhabitants: these were 0·28 for 1930, 1·04 for 1940 and 2·06 1950, and they had gradually fallen from this peak to a rate of 1·32 in 1959. The province of Foggia had higher rates—3·94 in 1940, 6·08 in 1950 and 3·63 in 1959.

This province has over 700,000 inhabitants and is among the largest in Italy. Extensive plains make up its central part, including its Adriatic coast to the north. In the east are the Gargano hills, and the highest incidence of human brucellosis. The foothills of the Apennines are in the west, and here human brucellosis shows the lowest incidence.

In his well-written and useful report, Tasselli deals with the topographical, geological, hydrographic, demographic, economic and environmental sanitation features of the area. He gives data, often tabulated in some detail, about epidemic and epizootic incidence and the numbers of cattle, sheep and goats in various districts and he discusses the conditions in which these animals are kept and handled.

Most of the human disease is due to *Brucella melitensis* and the author shows how the goats, to a much greater extent than either cattle or sheep, are responsible for epidemic and endemic spread. Goats are most numerous in and around towns and villages and they have been repeatedly found infected in association with human outbreaks, which mostly occur during April–June—the kidding season. In the goat, brucellosis infection is often latent and of long duration. The goats live in close contact with the inhabitants and are milked to give a direct (unpasteurized) supply to the public.

J. Cauchi

BOUVIER, G. Les possibilités de transmission de la brucellose du gibier. [**Possibility of Transmission of Brucellosis from Game**] *Schweiz. med. Woch.* 1961, July 15, v. 91, No. 28, 827–8.

The English summary appended to the paper is as follows:—

"In isolated cases, wild ruminants (roebuck, chamois) may be infected with brucellosis of the type Brucella

abortus. On the other hand, 5% of the hares on which autopsies are performed at the Institute Galli-Valerio are infected with Brucella suis, Danish variant, with generally very extensive lesions likely to prove the causative agents of brucellosis in humans."

WIEDERMANN, G. & REINHARDT, F. Eine neue serologische Methode zur Diagnose der Tularämie. **[About a New Serological Reaction for the Diagnosis of Tularaemia]** *Zent. f. Bakt.* I. Abt. Orig. 1961, v. 182, No. 1, 80–84, 1 fig.

The laboratory diagnosis of tularaemia has assumed considerable importance in the north-eastern part of lower Austria where the disease is enzootic among rodents.

A formal tube agglutination requires 24 hours and the slide agglutination of Henninger is insufficiently sensitive for early diagnosis when the serum agglutination titres are low. The authors have therefore used the preparation of an antigen-latex-particle suspension as introduced by Flamm and Wiedermann to obtain a reagent sufficiently sensitive to permit of rapid laboratory diagnosis.

A suspension of *Brucella tularensis* is broken down in a disintegrating apparatus and the supernatant is obtained after a short centrifugation. This should be brought to an optimal concentration which is determined by absorption at a wave length of 420 mμ in the spectrophotometer. It is mixed with a particulate latex in the proportion of 0·1 ml. latex to 2 ml. supernatant. Such preparations are stable up to 2 weeks. A mixture of 1 drop of such antigen preparations and one drop of serum dilution gives an appreciable separation of granules in 5–10 minutes and the titres recorded correspond to those obtained after 24 hours in a tube agglutination.

J. W. McLeod

SASLAW, S.; EIGELSBACH, H. T.; WILSON, H. E.; PRIOR, J. A.; CARHART, Sally. **Tularemia Vaccine Study. I. Intracutaneous Challenge** [SASLAW, EIGELSBACH, WILSON, PRIOR & CARHART]. *Arch. Intern. Med.* 1961, May, v. 107, No. 5, 689–701, 6 figs. **II. Respiratory Challenge** [SASLAW, EIGELSBACH, PRIOR, WILSON & CARHART]. *Ibid.*, 702–14, 9 figs.

The protective value of the available non-viable vaccines against tularaemia is not certain [this *Bulletin*, 1951, v. 26, 147] and the authors have used human volunteers, aged 21 to 35 years of age, from a State Penitentiary for the purpose of evaluating Foshay's killed tularaemia vaccine. 3 daily doses each containing 0·5 ml. were given subcutaneously to each volunteer; control volunteers were given a typhoid vaccine.

3 days later an intracutaneous injection of living *Brucella tularensis* (strain SCHU S 4) was given and the dose of 0·2 ml. was estimated to contain about 10 viable bacterial cells. 4 non-vaccinated volunteers received this challenge injection and all developed local lesions and regional lymphadenitis; 3 of the 4 had a systemic disturbance which was sufficiently serious to warrant streptomycin therapy; this was effective within 24 to 36 hours. 8 men who had been vaccinated with the typhoid preparation 3 to 8 weeks before also received the living tularaemia organisms; they responded in a similar manner to the non-vaccinated controls and the constitutional signs, which varied from severe to mild, warranted streptomycin therapy. The vaccinated group were challenged 19 to 44 days after vaccination and, all of 14 showed local lesions, with constitutional signs of infection in 4; 1 of the 4 showed signs of severe illness similar to that observed in the controls and all 4 required streptomycin therapy. 5 vaccinated men had a booster dose of vaccine 6 to 8 months later and when challenged, all showed local lesions and regional lymphadenitis and 2 showed constitutional signs of disease. 8 men who had been infected as controls and had been treated were also challenged, 2 and 8 months later; local lesions and regional adenitis occurred in all and 1 of the 2-month and 1 of the 8-month group showed systemic disease.

As judged by the methods described, the vaccine prevented generalized signs of the disease in most cases or delayed the onset of it. Re-infection was achieved in individuals who had previously been infected and this apparent lack of immunity may have been associated with prompt and early therapy. The vaccine did not prevent local infection and attention is drawn to the small dose necessary to infect—about 10 bacteria—and the dramatic cure afforded by streptomycin.

The second paper deals with similar volunteers and the administration of 10 to 50 viable tularaemia organisms by the respiratory route by means of a modified Henderson-type of apparatus. 6 of 8 controls receiving from 16 to 52 organisms by this route showed clinical signs of infection; 5 were given streptomycin and one tetracycline. Of 14 volunteers who had been vaccinated with killed vaccine 23 to 119 days previously, 8 developed clinical evidence of infection when given 13 to 57 living bacteria by the respiratory route; streptomycin or tetracycline was given and afforded relief of symptoms within 24 hours.

29 volunteers received a viable vaccine and mild local lesions occurred and disappeared within 2 to 3 weeks; about half had transient lymphadenitis. 18 of these were challenged by the respiratory route with doses from about 10 to 50 viable bacteria; 3 showed systemic evidence of infection compared with 8 out of 10 comparable volunteers who had not been vaccinated.

Attention is drawn to the small number of viable bacteria which caused clinical tularaemia when given by the respiratory route and to the results with the killed vaccine which did not protect against the infection given by this route; this should be compared with the results when infection was by the intracutaneous route in which the vaccine protected against systemic but not against local infection. The viable vaccine, which produced no untoward reactions gave significant protection against infection given by the respiratory route in the doses described.

R. Lovell

CURTIN, J. A., PETERSDORF, R. G. & BENNETT, I. L., Jr. ***Pseudomonas* Bacteremia: Review of Ninety-One Cases.** *Ann. Intern. Med.* 1961, June, v. 54, No. 6, 1077–1107. [53 refs.]

This is an analysis of 91 cases of *Pseudomonas pyocyanea* infection occurring in 88 patients in the Johns

Hopkins Hospital, Baltimore, between 1940 and 1959; the organism was isolated from the blood in all cases, either during life or immediately after death. The disease occurred mainly in infants and the elderly. In infants, prematurity and congenital abnormalities were prominent, in the aged debilitating illnesses, especially urological disorders. Another group of cases not confined to the extremes of life consisted of patients suffering from blood dyscrasias, mainly leukaemia. In adults the disease was manifested by the usual signs of blood infection—fever, rigors etc.; but in the infants there was often no fever but only general debility, failure to suck or thrive and often rapid deterioration. Umbilical sepsis was a factor of considerable importance in neonatal cases. In adults the disease was frequently associated with antibiotic therapy or prophylaxis for another condition. 73 of the 88 patients died: in 12 of the 73 the bacteraemia was cured but the patient died from the associated disease. Polymyxin B was the most effective antibiotic and the authors recommend that it should be given along with streptomycin; but response to treatment depended to a large extent on the underlying disease.

A. B. Christie

CLIFFORD, H. E. & STEWART, G. T. **Intraventricular Administration of a New Derivative of Polymyxin B in Meningitis due to *Ps. pyocyanea*.** *Lancet.* 1961, July 22, 177–80. [18 refs.]

Since 1957 the authors treated 5 infants (aged 1–6 months) with secondary meningitis due to *Pseudomonas pyocyanea* with a new preparation of polymyxin B—the sodium salt of polymyxin B methane sulphonic acid. This preparation appears to be free of the tissue-damaging effect of the sulphate of polymyxin B and the authors found no evidence of toxicity. Intramuscular injection of the drug alone did not produce satisfactory levels in the cerebrospinal fluid, and the drug was given intraventricularly as well in doses of 5,000 to 10,000 units at intervals of 2 to 3 days. Treatment may have to be continued for 4 weeks or more to avoid relapse; the 5 infants were cleared of infection within 11 to 35 days. The authors state that it is difficult to assess possible neurological sequelae of the meningitis and the neurosurgical procedures carried out as treatment of the original conditions for which the infants were admitted to hospital.

A. B. Christie

HALL, M. R. P. **Leptospiral Fever.** *Postgraduate Med. J.* 1961, Apr. v. 37, No. 426, 210–12, 1 fig. [12 refs.]

The author reports a non-fatal illness, which presented in a manner very similar to that of classical Weil's disease, in which the serological examinations [up to about the 21st day of disease] suggested that *Leptospira canicola* was the cause. Jaundice, oliguria and raised blood urea—all features of this case—are very unusual manifestations of canicola leptospirosis.

There is a great variety in the clinical response to leptospiral infection. Short febrile illnesses with or without meningeal and respiratory tract involvement may be misdiagnosed as atypical pneumonia, virus pneumonia, or influenza. Various names which have been given to leptospiral illnesses in different parts of the world tend to be confusing and the need for a simple clinical classification is suggested; moreover "Weil's disease", if retained, should be reserved for "severe", "malignant" or "icteric" forms regardless of the responsible serotype

L. H. Turner

ANTUNOVIC-MIKACIC, S. Noviji epidemiološki problemi leptospiroza. [**New Epidemiological Problems of Leptospirosis**] *Higijena.* Belgrade. 1960, v. 12 No. 4, 388–406, 2 graphs & 3 maps. [35 refs.] English summary.

In the winter of 1958 the author isolated 22 strains of *Leptospira icterohaemorrhagiae* from 72 rats killed by poison in Belgrade, although only 1 case of human leptospirosis was known to have occurred in the city in the same year. The incidence of leptospire infection was high in large domestic animals (38·8% of 711 pigs, horses cattle and sheep) examined serologically between 1950 and 1956; 18 of 192 (9·3%) dogs examined were seropositive, mostly to *L. sejroe, L. canicola* and *L. pomona* The annual number of recorded human cases of leptospirosis in Yugoslavia has risen during the last decade from 12 cases in 1949 to 224 in 1958, with a maximum incidence of 1,037 cases in 1955 and 564 in 1956. Of 443 cases investigated in 1957 and 1958, 183 (41·2%) were due to *L. pomona*, 93 (20·9%) to *L. sejroe*, 47 (10·6%) to *L. grippotyphosa*, 28 (6·3%) to *L. icterohaemorrhagiae* and 20 (4·5%) to *L. mitis.*

D. J. Bauer

ZONCHELLO, S. A. Rilievi sulla infezione tetanica nella Provincia di Cagliari nel decennio 1948–1958 [**Observations on Tetanus Infection in the Province of Cagliari in the 10-Year Period 1948–1958**] *Arch. Ital. Sci. Med. Trop. e Parassit.* 1961 Apr., v. 42, No. 4, 198–208. [10 refs.]

The hospitals of Cagliari, Sardinia, treated 120 patients for tetanus between January 1st, 1948 and December 31st 1958 [which is a period of 11 rather than 10 years] Tetanus was only made notifiable in 1955 but it is unlikely that there were any other cases in addition to those included in this series, as no other hospitals exist to which they could have gone. 88 were men and 32 women, and 15—nearly half—of the latter died as compared with 25—under 30%—fatal cases among the men. 26 occurred in the first 5 years of life, 37 patients were aged 6–10 years, 26 were 11–20 and 31 were all over 20. The author presents his data in a number of tables and comments on traumatic, occupational and even "climatic" [? weather] factors. He also analyzes the clinical manifestations which have been recorded in this series of tetanus cases.

J. Cauchi

RYAN, B. **The Treatment of Tetanus in Children with Chlorpromazine.** *Papua & New Guinea Med. J.* Port Moresby. 1961, Mar., v. 5, No. 1 27–9.

The author used chlorpromazine in the treatment of two patients with tetanus, one a neonate and the other

a child of 5. The neonate was given 5 mgm. orally, later increased to 10 mgm., every 6 hours, the older child 40 mgm. every 3 hours, later reduced to 25 mgm. every 4 hours. The neonate died after 5 days' treatment, the older child recovered after 21 days. Both patients were also given phenobarbitone and mephenesin. The author does not claim that these 2 cases prove a great deal but feels that chlorpromazine is a useful drug in the treatment of tetanus. His comments on the use of the drug in gastro-enteritis of infants are interesting—that 1 mgm. per lb. body weight in a moderately dehydrated infant will produce extreme drowsiness, in a normal infant the same dose will have no effect, but in a severely dehydrated child the dose is excessive. *A. B. Christie*

VIRUS INFECTIONS

The order in this section is:—***General, Psittacosis-Lymphogranuloma group, Pox group, Respiratory group, Reovirus group, Enteric group, Arthropod-borne group, Others.***

HERRLICH, A., DIESFELD, H. J. & SCHMIDT, H. Die Pockenerkrankungen in Ansbach 1961. [**The Smallpox Epidemic in Ansbach in 1961**] *Deut. med. Woch.* 1961, July 28, v. 86, No. 30, 1413–20, 1423–4, 10 figs. (5 on 2 pls.)

This is a full account of an epidemic of 4 cases of smallpox which occurred in Ansbach in 1961. The infection was introduced by a young man 28 years of age who arrived home on March 11th from a visit to India lasting some weeks. He was taken ill with pains in the head and neck on March 18th and treated with antibiotics 2 days later for a reddening of the throat. On March 23rd–24th red spots appeared on the hands and forearms which extended to the shoulder and vesicles were seen in the mouth. His general condition had now improved but because of the history of his visit to India he was transferred on March 25th to isolation in hospital with the diagnosis of varicella. Ultimately on March 28th the experts who had been called in diagnosed modified smallpox (varioloid) confirmed by serological examination. This patient, who had vaccination scars, made a complete recovery.

The mother of this man had also been vaccinated and was revaccinated on March 31st but was taken ill on April 4th and died 7 days later from haemorrhagic smallpox. The other 2 cases of smallpox were in the father of the young man and in the doctor who had attended the latter. Both these patients suffered only from the modified disease and recovered. All of the 4 patients had been vaccinated.

There is a full clinical account of these 4 cases and of the results of the serological and virological tests which were carried out.

The measures taken to limit the spread of the infection which included the vaccination of more than 15,000 persons of the population are fully described with a lengthy discussion.

No further cases of smallpox occurred.

M. E. Delafield

SWORD, J. M. **Smallpox in Central Province, Nyasaland.** *Brit. Med. J.* 1961, July 15, 165–6, 3 figs.

Smallpox is endemic in Nyasaland and from time to time assumes epidemic proportions. For a long time, attempts have been made by vaccination campaigns, to control, if not to eradicate, the disease. In the year 1960, especially in the Central Province, much political opposition to vaccination arose, and towards the end of that year the number of cases of smallpox known, and requiring isolation, necessitated extraordinary measures. During a period of 5 months 620 patients were admitted to specially assigned accommodation; of these patients 95 died. No estimate could be made of the number of cases, and the number of deaths, which had been concealed, but there must have been many. It is recorded that "cases of variola minor accounted for roughly 70% of the total" [but the grounds on which the differential diagnosis between the major and the minor forms of the disease was made are not given].

Diagnosis did not present any great problem. Onset was usually with fever and general weakness for 3–5 days, followed by the rash. Antibiotics failed to shorten the course of the disease, but exercised a favourable control on bronchopneumonia, the commonest complication in the younger age-groups.

Involvement of the eye, especially the conjunctiva, occurred in many patients, sometimes involving loss of sight.

Opposition to vaccination ceased in October and the programme could proceed, but "by this time the damage was done".

J. Pickford Marsden

PATNAIK, K. C. **A Study of the Recent Rise of Smallpox Cases in the Madras City.** *Indian J. Pub. Health.* 1960, Oct., v. 4, No. 4, 206–16.

In the city of Madras—a recognized endemic centre of the disease—the incidence of smallpox has remained consistently high since 1957. The present investigation was instigated by the Director General of Health Services, New Delhi, partly because it was being reported that the disease was occurring even among persons supposed to have been re-vaccinated on several occasions. The author, an Assistant Director General of Health Services, from his study of the situation reached the conclusion that the problem was the administrative one of ensuring successful primary vaccination and systematic re-vaccinations at definite intervals of time with active, potent lymph.

It was found that in the majority of instances cases occurring in re-vaccinated subjects could be ascribed to failure of the re-vaccination to "take". Many patients were encountered who either had not been vaccinated or

had been vaccinated or re-vaccinated during the incubation period; or in whom immunity had waned over a long period since primary vaccination.

J. Pickford Marsden

FALISEVAC, J., ARUMANAYAGAM, P., SIVAGANARATNAM, K., SIVAGANAM, C. & PADLEY, R. **A Smallpox Outbreak at Leyden Island, Ceylon, 1958.** *Ceylon Med. J.* (n.s.) 1960, Dec., v. 5, No. 4, 183–94, 2 pls. and 1 folding graph.

This paper describes in considerable detail an outbreak which comprised 27 known cases. There were two deaths —from purpura variolosa.

Although Ceylon lies close to areas where smallpox is endemic the Island itself is normally clear of the disease which, however, is imported from time to time *via* the trade routes with infected countries or by illicit immigration which evades the quarantine measures.

In the present outbreak the source of the infection was not determined. The first case was probably that of a 49-year-old man in a harbour town which provides frequent contacts with the south-eastern and southern coasts of India. He died of a haemorrhagic illness. The disease affected mainly only close, chiefly family, contacts. The usual diagnostic difficulties were encountered—in haemorrhagic cases, especially at the commencement of an outbreak, and in some mild cases modified by previous vaccination which some medical officers are reluctant to query as smallpox and too readily accept as chickenpox.

Towards the end of the outbreak some difficulty was also met with in successfully vaccinated contacts. In 5 such cases sparse focal rashes were diagnosed as vaccinial.

In general the results of the available virological investigations were not very helpful.

J. Pickford Marsden

GRIST, N. R. **Laboratory Diagnosis of Smallpox.** *Health Bull.* (Issued by the Chief M.O., Dept. of Health, Scotland.) 1961, July, v. 19, No. 3, 46–7.

"Outbreaks of smallpox are a possible danger in an incompletely vaccinated community such as Britain. Such outbreaks usually originate from an infected person from abroad entering the country during the incubation period of illness. Spread of infection is more likely if this primary case is of modified and atypical character, as may occur if there is residual but incomplete immunity from vaccination some years previously. It is therefore desirable that all cases of *possible* smallpox should be investigated virologically. The principles of laboratory diagnosis and methods of collection and transport of specimens are described above."

FIORILLO, A. M. & CAMPANA, C. L. Perfil electroforético das proteínas séricas e proteína C-reativa na varíola. **[Electrophoretic Pattern of Serum Proteins and of C-Protein Reaction in Smallpox]** *Rev. Inst. Med. Trop. São Paulo.* 1961, Mar.–Apr., v. 3, No. 2, 85–90, 8 figs.

The English summary appended to the paper is as follows:—

"During the outbreak of a smallpox epidemic in th area of Ribeirao Prêto (SP), the electrophoresis of th serum proteins and the C-protein reaction were performe in material from 29 patients in different stages of th disease.

"The diagnosis of the disease was based on th classical clinical picture: in 3 of the patients th diagnosis was confirmed by the culture of virus in th chorio-allantoic membrane of chick embryo.

"No change was found in the albumin fraction; th α_2 globulin was increased in the stages of vesicle an crusts, when exudative reaction of the skin lesions wa present. The γ-globulin increased but less than the fraction, during the recovery of the skin lesions.

"The positivity of the C-protein reaction was parall with the increase of the α_2 globulins (higher frequenc and intensity during the vesicle phase).

"A patient with nephrosis behaved as the oth patients, although the increase of the γ-globulin was n so clear as in the other cases."

WEBER, G. & LANGE, J. Zur Variationsbreite der "I kubationszeiten" postvakzinaler zerebraler Erkra kungen. **[The Range of the "Incubation Period of Post-Vaccinal Cerebral Damage]** *Deut. me Woch.* 1961, Aug. 4, v. 86, No. 31, 1461–8, 149 4 figs. [85 refs.]

A definite diagnosis of a causal relationship betwee smallpox vaccination and a following acute inflammato disease of the central nervous system sometimes presen great difficulty.

The work described in this paper had as its purpo the determination of the incubation period and consiste of a careful analysis of the clinical histories of 265 cas of post-vaccinal cerebral disease in which autopsy ha been carried out. Records of these cases were obtaine from various sources, but cases of fulminating vaccin encephalitis in which death occurred in a very sho time after vaccination were excluded from the analys

The methods adopted for the classification and statis cal assessment of the data are stated and the results a described with tables. The incubation period was defin as the period from the day after vaccination to the da on which the first definite neurological sympto occurred. This incubation period was found to have range of 4 to 18 days and it is considered that neur logical symptoms which appear outside this range cann be regarded as being related to the vaccination. Pos vaccinal encephalitis which occurs almost exclusively the first 2 years of life was found to have an incubatio period of $8{\cdot}6 \pm 2{\cdot}3$ days. Post-vaccinal encephalomyelit which is found mainly in children over 2 years of age an in adults, had an incubation period of $12{\cdot}3 \pm 2{\cdot}1$ day The day of death in encephalitis was found to be on a average $10{\cdot}5 \pm 3{\cdot}0$ days with a maximum of 20 days aft the day of vaccination: the corresponding figures encephalomyelitis were $16{\cdot}3 \pm 3{\cdot}9$ days with a maximu of 32 days.

M. E. Delafield

EPP, Christine. Über das Antikörperbild des Menschen bei normalem und gestörtem Verlauf einer Pockenschutzimpfung. [**The Antibody Picture After Normal and Complicated Reactions to Vaccination**] *Arch. f. Hyg. u. Bakt.* 1961, June, v. 145, No. 4, 256–73.

Here reported are the results of serological tests to determine the antibody "picture" after smallpox vaccination when there is a normal reaction and also when there follows a reaction complicated in particular by encephalitis. In addition these tests were made after a pre-vaccination administration of formalin-inactivated vaccine (Herrlich) which has been devised for use on children who may, on account of age or other causes, react abnormally to ordinary vaccine.

Details are given of the methods and vaccine materials employed in the tests of haemagglutination and its inhibition, complement-fixation, precipitin content and virus neutralization.

The results, fully described with tables, are followed by a careful discussion.

The main observations were:—(1) after primary successful vaccination neutralizing and haemagglutination inhibiting antibodies are regularly produced by the 10th day and remain in the blood for years. In little children, however, complement fixation and precipitin formation could only rarely be demonstrated, (2) the antibody response of adults and older children to re-vaccination differs from that of small children to primary vaccination in certain respects—the titre of neutralizing antibodies is higher and complement fixation and precipitin tests are more frequently positive, (3) pre-vaccination with vaccine virus antigen does not as a rule bring about antibody formation but the subsequent ordinary vaccination is followed by an antibody reaction like that observed in re-vaccinations and (4) examination of the sera of children who had suffered from post-vaccinal encephalitis showed that in these precipitins had usually been produced. *M. E. Delafield*

PHILIP, R. N.; BELL, J. A.; DAVIS, D. J.; BEEM, M. O.; BEIGELMAN, P. M.; ENGLER, J. I.; MELLIN, G. W.; JOHNSON, J. H.; LERNER, A. M.; VOGEL, J. E.; JENSEN, D. V. **Epidemiologic Studies on Influenza in Familial and General Population Groups, 1951–1956. II. Characteristics of Occurrence** [PHILIP, BELL, DAVIS, BEEM, BEIGELMAN, ENGLER, MELLIN, JOHNSON & LERNER]. *Amer. J. Hyg.* 1961, Mar., v. 73, No. 2, 123–37, 3 figs. [18 refs.] **III. Laboratory Observations** [DAVIS, PHILIP, BELL, VOGEL & JENSEN]. *Ibid.*, 138–47. [15 refs.] **IV. Vaccine Reactions** [BELL, PHILIP, DAVIS, BEEM, BEIGELMAN, ENGLER, MELLIN, JOHNSON & LERNER]. *Ibid.*, 148–63, 2 figs. [28 refs.]

II. This report presents epidemiological observations on 3 influenza outbreaks which occurred during 5 years of study 1951–56 in localized areas in Maryland. In 1951–52 observations extending over 19 weeks covered 5 local areas comprising 616 households with 3,077 persons and the study became progressively more extensive so that in 1955–56 there were 32 weeks of observation in 26 local areas with 4,078 households comprising 18,822 persons. On entering the study individuals were given monovalent influenza vaccines and without revaccination were observed during subsequent years for acute febrile respiratory illness (AFRI). The latter was defined as illness of sudden onset with respiratory symptoms lasting 2 or more days and accompanied by a pyrexia of 99°F. or more. Virus isolation was the chief method of confirming a clinical diagnosis and throat swabs and garglings were collected in beef-heart infusion broth or skim milk from both ill persons and close associates.

Outbreaks of influenza B extended over 7 weeks in both 1952 and 1955 and an outbreak of influenza A over 9 weeks in 1953. An increase in AFRI was associated with an increase in virus isolation; virus isolations were not successful in inter-epidemic periods. During each influenza B outbreak, attack rates in the school age-groups (6–17 years) were higher than in younger and older groups. During the type A epidemic, attack rates were similar in the pre-school and school age-groups and both were higher than the rate in adults. Observations on 137 households from which type A virus was isolated in 1953 and 137 households from which type B virus was recovered in 1952 and 1955 showed that AFRI occurred within 10 days of onset of the first virus-positive case. Furthermore, 62 of 64 type A virus-positive AFRI and 29 of 31 type B positive AFRI occurred among household associates within 10 days of the initial virus positive case. There were, respectively, 648 and 763 persons residing in households positive for type A and B virus; type A virus was isolated from 73% and type B from 59% of AFRI tested, so that it seemed that nearly all of the AFRI associated with a virus-positive household case were influenza. Type A virus was isolated from 38% and type B from 11% of the few afebrile illnesses tested. Type A virus was recovered from 2 of 40 persons without illness but type B was never recovered from healthy persons; thus afebrile cases and inapparent infections occurred but were considerably less frequent than febrile cases.

Schoolchildren most frequently introduced influenza into the household; this was particularly so during type B epidemics. Further, it was shown that for both virus types there was a decrease in the secondary AFRI attack rate with increase in age, probably reflecting acquisition of immunity as a result of prior experience with influenza. It was interesting too that there were fewer instances of AFRI in persons with high haemagglutination-inhibition antibody titres to both types than in those with low titres. Finally, there was no evidence that persons infected with influenza B in 1952 were resistant to influenza B infection in 1955, no doubt owing to the change in antigenic pattern of influenza B virus between 1952 and 1954.

III. Serological and virus isolation studies are presented. Serum samples were obtained by diluting 0·2 ml. of blood, from a finger prick, with physiological saline to give a dilution of 1/20. Haemagglutination-inhibition (HI) antibody responses at various intervals after vaccination were compared to the pre-vaccination response. Monovalent adjuvant influenza A and B vaccines each containing 100 chick cell agglutination

(CCA) doses produced a greater number of 4-fold HI antibody responses to homologous antigens than did monovalent aqueous vaccines (300 CCA units). These antibody responses to adjuvant vaccines persisted for at least 3 years in 60% of individuals given influenza A vaccines and in 95% of those given B vaccines, although it must be noted that these persons were exposed to an epidemic of influenza B in 1952 and influenza A in 1953. 4-fold responses to heterologous influenza A antigens after inoculation with adjuvant vaccines were far less frequent; this, however, was not so with heterologous B antigens. The number of 4-fold homologous antibody responses detected by egg neutralization was of the same order as detected by HI but complement fixation detected only about one-third of such responses following vaccination with A adjuvant and one-tenth following vaccination with B adjuvant.

There was no significant difference in the virus recovery rate from throat washings and from throat swabs. 21 of 27 samples that had yielded virus were positive again 7 months later despite refreezing. Influenza A was more readily recovered from specimens than B virus and most frequently during the first 3 days of illness. Influenza A virus was also isolated 1–2 days before the onset of fever in 2 of 3 samples tested and as long as 9 days after onset in 3 of 14 samples tested. Finally, about 90% of persons who were ill and from whom A or B virus was isolated and 90% of ill persons living in households with such individuals showed a 4-fold HI antibody response; virus was isolated from about 70% of those persons with antibody response to influenza A and about 40% of those with an antibody response to influenza B.

IV. Between 1951 and 1956 about 18,000 persons were given mineral oil adjuvant or aqueous influenza vaccines and were observed for systemic, allergic and local reactions. Adjuvant vaccines were given subcutaneously in the deltoid region. Information on systemic and allergic reactions was obtained on 9,755 persons. Assessment of systemic reactions (all departures from normal health having onset within 48 hours after vaccination and not localized to the site of vaccination) was difficult because there was no placebo group; nevertheless, febrile non-respiratory syndromes occurred with high frequency (40% of children under 6 years of age receiving polyvalent A and B vaccine) in persons receiving the monovalent type B and polyvalent aqueous vaccine products. The frequency of such reactions decreased with increase in age and was directly related to the quantity of virus administered per dose of vaccine. On the other hand, mineral oil adjuvant vaccines caused systemic reactions in only 1% of subjects.

Maculopapular eruptions, urticaria, periorbital oedema and asthmatic attacks were considered to be allergic and were noted in 43 instances only. The majority of such reactions occurred within 48 hours of vaccination, were mild and of short duration and were distributed equally among persons receiving aqueous and adjuvant products. Early local reactions were studied in 3,192 schoolchildren. As with systemic reactions, early local reactions (erythema and induration $\geqslant$ 15 millimetres in diameter) occurred more frequently with aqueous (30–60% of subjects) than with adjuvant vaccines (2%)—this was probably due to the subcutaneous administration of aqueou vaccines; such reactions occurred particularly with typ A rather than type B aqueous vaccine. The mos troublesome reaction to adjuvant vaccines was th occasional occurrence of chronic granulomas whic varied from a small nodule to a mass 9 cm. in diameter The larger masses commonly developed into "cysts or "sterile abscesses" which usually ruptured spontaneously; they developed between 3 and 27 month after vaccination and were found in only 24 subject (0·22%), predominantly Negroes and young children.

[For part I see this *Bulletin*, 1954, v. 29, 795.]

D. Taylor-Robinson

DAVIDSON, W. S. **Vaccination in Influenza: Publi Health Aspects.** *Med. J. Australia.* 1961, Ma 25, v. 1, No. 12, 447–9. [11 refs.]

This paper reports some data on an influenza epidemi caused by an A/Asian virus strain in Western Australi in 1959, in areas previously involved in the 195 pandemic, and in which polyvalent influenza vaccine including Asian strains had been used widely.

In a questionnaire survey of some 10,000 office worker and hospital staff the influenza attack rate in 1959 wa shown to be 35·6% in the unvaccinated, compared wit 34·3% in those vaccinated in 1958 and 36·9% in peopl last vaccinated before 1958.

Of those vaccinated in 1959, the case rate in thos previously vaccinated was 23·4% or 28·5% dependin whether the booster dose was given before or during th epidemic. In those not previously vaccinated the cas rate was 33·6% when vaccine was given before th epidemic, and 41·7% when given during the epidemic.

The small difference between vaccinated and un vaccinated groups, as compared with the 1957 experience probably reflects the increased frequency of naturally immune people in the community. In the group vaccinated for the first time during the epidemic the cas rate was actually higher than in the unvaccinated group This, the author believes, may be due to the polyvalen vaccine stimulating recall of antibodies to pre-Asia influenza strains at the expense of a serological respons to the Asian strain itself. *Derek Hobson*

NEUBERT, G. Cytomorphologische Befunde bei de Resorption von Influenza-Depotimpfstoffen. **[Cyto morphological Findings in the Absorption o Influenza Depot-Vaccines]** *Zent. f. Bakt.* I. Ab Orig. 1961, v. 182, No. 3, 281–93, 12 figs. [1 refs.]

MCLEAN, D. M., ROY, T. E., O'BRIEN, M. J., WYLLI J. C. & MCQUEEN, E. Joan. **Para-Influenz Viruses in association with Acute Laryngo tracheobronchitis, Toronto, 1960–61.** *Canadia Med. Ass. J.* 1961, Aug. 5, v. 85, No. 6, 290–9 [25 refs.]

"Between November 1, 1960, and January 28, 196 virological and bacteriological studies were carried ou

on 155 children, including 60 under two years of age, who were hospitalized with acute laryngotracheobronchitis. Isolation of Para-influenza-1 virus from 73 children, 17 of whom showed serological evidence of infection with Para-influenza-1 virus, and isolation of Para-influenza-3 virus from seven children, together with serological evidence of infection in three of these, suggested strongly an etiological association between these viruses and tracheitis."

PARKER, W. L., WILT, J. C. & STACKIW, W. **Adenovirus Infections.** *Canadian J. Pub. Health.* 1961, June, v. 52, No. 6, 246–51, 3 figs. [16 refs.]

The biological characteristics of the adenovirus are briefly reviewed and an attempt made to classify on clinical and epidemiological grounds the 18 types occurring in man: types 3, 4, 7, 8 and 14 produce epidemic disease, the others tend to occur sporadically. Criteria for diagnosing adenovirus infection, with varying degrees of certainty, are discussed and an account given of the illness in 70 patients. Adenovirus was isolated from 42 of these patients, the illness was diagnosed serologically in the remainder: 35 of the 42 isolates were adenovirus type 3, and 25 were from children under 1 year old. There were 5 fatal cases, type 3 virus being isolated from post-mortem lung tissue and bronchial secretions in 4 of the 5. The post-mortem lung tissue showed a necrotizing bronchiolitis with intranuclear and intracytoplasmic inclusion bodies. The authors feel that adenovirus infection, especially in children, is so serious that the more frequent use of a polyvalent adenovirus vaccine should be considered. *A. B. Christie*

MCCLELLAND, Laurella; HILLEMAN, M. R.; HAMPARIAN, V. V.; KETLER, A.; REILLY, C. M.; CORNFELD, D.; STOKES, J., Jr. **Studies of Acute Respiratory Illnesses caused by Respiratory Syncytial Virus. 2. Epidemiology and Assessment of Importance** [MCCLELLAND, HILLEMAN, HAMPARIAN, KETLER, REILLY, CORNFELD & STOKES]. *New England J. of Med.* 1961, June 8, v. 264, No. 23, 1169–75. **3. Clinical and Laboratory Findings** [REILLY, STOKES, MCCLELLAND, CORNFELD, HAMPARIAN, KETLER & HILLEMAN]. *Ibid.*, 1176–82, 1 fig. [10 refs.]

Between January 1959 and June 1961 667 children with acute respiratory disease and 153 controls were studied at two children's hospitals in Philadelphia. Mostly the patients were under 5 years of age and most were not admitted to hospital. Infection with respiratory syncytial virus RSV (or CCA) was diagnosed by complement-fixation tests, combined, in some cases with neutralization tests and virus isolation. There was no marked epidemic prevalence of respiratory infection during any particular period. Infection with the virus was mostly detected in March, April and May 1960; the infection was found in 59 of 408 cases of upper respiratory infection, in 17 of 52 cases of bronchitis, in 10 of 26 cases of bronchiolitis, in 22 of 56 cases of bronchopneumonia and in 1 of 11 cases of croup. There were 3 infections in the 153 controls. About 1 in 5 of all patients under the age of 5 were apparently infected with RS virus. Antibody levels in older patients were higher than those in younger patients and there was evidence that most children acquired antibody by infection during the first 4 years of life, and in the older age-groups there were less virus-positive cases. 67% of adults possessed antibodies. Virus was isolated from 21 of 34 patients in whom an antibody rise was detected.

In the second paper detailed clinical findings of 109 cases are tabulated and discussed and illustrated by representative case records. The basic syndrome was "severe rhinitis, cough and fever, with relatively little or no significant pharyngitis and conjunctivitis". Mild catarrhal conjunctivitis and otitis media were seen and also mild cervical lymphadenopathy and vomiting. Bacterial infections were not apparently seen and no apparent improvement was produced by antibiotic therapy.

It is pointed out that the disease most closely resembles influenza apart from the rather severe rhinitis and the frequency of lower respiratory tract signs. Fever and cough were more frequent than in "common colds".

[The results show the aetiological importance of RS virus and should be compared with those of CHANOCK *et al.* (see p. 1195) with which they disagree on points of detail. The papers should be read in the original by those interested in the subject.] *D. A. J. Tyrrell*

PAUTRIZEL, R., SZERSNOVICZ, F. & MASSAL, R. Recherche des anticorps antipoliomyélitiques par la méthode de séro-neutralisation dans la population du Sud-Ouest de la France. [**Serum Neutralization Tests for Poliomyelitis Antibodies in South-West France**] *J. Méd. de Bordeaux.* 1961, June, v. 138, No. 6, 729–36, 3 figs.

This is a short account of the results of the testing of 1,043 sera from persons of ages ranging from a few months to many years, living in Bordeaux and its surroundings, for the presence of antibodies to the 3 antigenic types of poliomyelitis virus by the method of LÉPINE *et al.* [this *Bulletin*, 1960, v. 35, 93]. All the sera were from subjects who had never been vaccinated against or suffered from poliomyelitis.

The findings are described with tables of results and discussed with comparison of observations made elsewhere in France.

Antibodies were found present against the 3 antigenic types to about an equal extent—74·5% against type 1, 70·6% against type 2 and 69·4% against type 3. Particulars are also given of the percentages of sera containing antibodies to all 3, or 2 or 1 of the 3 types.

Immunity of the population as judged by the results of these serum tests by age, rises rapidly in infancy, then more slowly attaining a stationary peak at about 25 years of age. These results were obtained in tests in which the sera were undiluted: using 1 in 10 dilutions of serum reveals peaks in the immunity at 6–8 years and at 24–28 years. *M. E. Delafield*

MÉCS, I., SZÖLLÖSY, E., KUKÁN, Eszter & BÉLÁDI, Ilona. **Aetiological and Epidemiological Studies of Poliomyelitis in Szeged, 1956–1959.** *Acta Microbiologica.* Budapest. 1961, v. 8, No. 1, 15–19. [15 refs.]

This is a brief account of the isolation of enteroviruses in Hungary in the years 1956–59. Epidemics of poliomyelitis occurred in Hungary in 1957 and 1959 and during these years type 1 poliovirus was isolated from most of the paralytic cases examined. In "abortive" cases poliovirus and other enteroviruses were isolated in approximately equal numbers. A small serological survey in 1957 and 1959 showed that antibodies to type 1 poliovirus were very prevalent even in young children (81% of 31 children 3–7 years old in 1959), probably due to the epidemic of type 1 virus. Antibodies were rare to types 2 and 3, being present in 55% and 42% of children 3–7 years old in 1959. *A. J. Beale*

RUDNAI, O. **The 1959 Poliomyelitis Epidemic in Hungary.** *Acta Microbiologica.* Budapest. 1960, v. 7, No. 4, 433–44, 6 figs. [15 refs.]

In Hungary before the war epidemics of poliomyelitis recurred at intervals of 5–6 years, but in the period 1954–59 there were 4 epidemic years, type 2 being at first the main strain, then type 3, and finally, in 1957 and 1959, type 1. The high pathogenicity of the type 1 strain was probably a main cause of the magnitude of the 1959 epidemic, when there was an attack rate of 18·3 per 100,000. Nevertheless both the incidence and mortality seemed to be considerably influenced by the Salk vaccination carried out in late infancy since 1957 and energetically conducted as a campaign in face of the epidemic. This was shown by the rapid drop of the epidemic, especially in Budapest where the vaccination had begun earlier, and by the marked decrease in the attack rate at 1–2 years, compared with that of previous epidemics. Moreover, the case fatality rate for the patients immunized with Salk vaccine was 1·8% in contrast to 4·6% for the unvaccinated patients. *H. Stanley Banks*

SEAL, S. C. & GHARPURE, P. V. **Notes on an Outbreak of Poliomyelitis in the Andaman Islands, 1957.** *Bull. World Health Organization.* Geneva. 1961, v. 24, No. 1, 123–7.

The first known major outbreak of poliomyelitis in Port Blair, South Andaman Island, occurred in the first few months of 1957, when 32 cases, all in children and all paralytic, were detected; almost 80% were under 3 years of age; the majority had lower limb paralysis and there were 2 bulbar cases.

This report gives the results of neutralization tests for poliomyelitis antibodies in 132 sera, from patients, recent contacts, and non-contacts of recent cases; 97% of those showed evidence of immunity to at least one poliomyelitis type, and 83% had antibodies to all 3 types. Poliomyelitis infection is therefore highly endemic in this island. Of the 13 sera from patients examined 6 showed antibody only to type 1 virus and 7 to types 1, 2, and 3. The 1957 outbreak was therefore presumably caused b type 1 virus attacking a well-salted community.

It is thought that a more intensive study in th interesting region might yield important epidemiologic information. *H. Stanley Banks*

LIE-KHING-TING. **Poliomyelitis in Indonesia. A Ser logical Survey for Neutralizing Antibodi against Polio Viruses 1957–1959.** *Trop. & Ge graph. Med.* Amsterdam. 1960, Dec., v. 12, No. 293–302, 3 figs. [13 refs.]

A serological survey of the incidence of poliovir infection in Bandung, Indonesia, is reported. It was foun that antibodies to all 3 types were very prevalent i adults and school children, so 202 pre-school childre attending Mother and Child Health Clinics wer sampled. 50% had type 1 and 2 antibodies by 3 yea of age and type 3 by 1 year. Clinical poliomyelitis rare in Indonesia but presumably as standards improv the frequency will increase. It is recommended tha when the level of infant mortality drops, 100 seru samples from 6- and 3-year-old children should be take and tested for antibodies. If these tests show deficiencie in antibodies a vaccination programme may be require At present poliovaccination is not required excep possibly for more wealthy communities though, eve here, serological evidence of the necessity has not bee produced. *A. J. Beale*

OZERE, R. L. & ROZEE, K. R. **A Survey of Polioviru Neutralizing Antibody in Newfoundland, 1959 60.** *Canadian Med. Ass. J.* 1961, July 8, v. 8 No. 2, 63–7, 3 figs. [16 refs.]

Only 2 authenticated outbreaks of poliomyelitis hav been recorded in Newfoundland, one in 1953 and anothe in 1959. The main brunt fell on pre-school age childre in both epidemics. The survey of antibodies showed tha the incidence and rate of acquisition was intermediat between the pattern of areas where the virus is prevalen and disease rare (*e.g.* Japan, Morocco, or Cairo) an areas where epidemics occur (Munich, Iceland, or Miami *A. J. Beale*

WEHRLE, P. F.; REICHERT, R.; CARBONARO, O.; HAGEN F.; DAY, P. A.; WHALEN, J. P.; PORTNOY, I **Transmission of Polioviruses. I. Spread o Naturally Occurring Poliovirus Type I in Partially Immunized School Population** [WEHRL REICHERT & CARBONARO]. *Pediatrics.* Springfiel Ill. 1961, May, v. 27, No. 5, Pt. 1, 748–54. [1 refs.] **II. Spread of Attenuated Poliovirus Typ III in a Partially Immunized School Populatio** [WEHRLE, HAGEN & CARBONARO]. *Ibid.*, 755–6 **III. Prevalence of Polioviruses in Pharynge Secretions of Infected Household Contacts o Patients with Clinical Disease** [WEHRLE, CA BONARO, DAY, WHALEN, REICHERT & PORTNOY *Ibid.*, 762–4. [12 refs.]

3 aspects of the spread of polioviruses are considere in these 3 papers. The first 2 concern studies at a summ vacation school in McLean, New York State.

I. In the first year, after a case of paralytic poliomyelitis occurred in a vaccinated subject, 75 (out of 150) school children and 24 young siblings were studied. 60% had been given 3 doses of Salk vaccine but, even so, many children still lacked antibodies to types 1 and 3 viruses. In all there were 2 cases of paralytic poliomyelitis and 43% became infected, 50% of these excreting virus from the pharynx. Prior immunization did not prevent the carriage of virus to siblings nor their sensitivity to such family contact infection. On the other hand, children of 5–9 years who had had 3 or 4 doses of Salk vaccine were infected significantly less commonly than unvaccinated children of the same age.

II. At the end of the first year's study described above 60% of the children lacked antibodies to type 3 poliovirus. The next year it was decided to administer 1 million TCD50 of attenuated type 3 poliovirus vaccine to 6 children who had no antibodies to type 3 poliovirus in the previous year. There were 106 people in the second study, 44 of whom had been in the first one. It was found that at the commencement of the second study only 7 out of 95 people lacked type 3 antibodies. This change was ascribed to vaccination between seasons with potent vaccine because rises in titre only occurred in the vaccinated and no type 3 poliovirus isolations were made in the area during the interval. All 6 vaccinated children excreted virus for at least 1 week and usually for at least 2½ weeks. Only 3 out of 25 family contacts became infected and all 3 had had killed poliovaccine and had high titres of antibody. 1 out of 3 close school contacts became infected. This child had only a low titre of antibodies to type 3 whereas the other children all had high titres. There were another 5 isolations of type 3 poliovirus from more remote contacts but no isolation from sewage. No isolations were made from the pharynx. In the previous year type 1 poliovirus predominated, only 2 isolations of ECHO 9 being made in addition. The second year there were 40 other viruses isolated, mainly Coxsackie B2 and 3. The explanation for the more limited spread of the attenuated type 3 than the virulent type 1 strain is thought to be a quality of the virus. Alternative explanations that high titres of neutralizing from Salk vaccination or prevalence of other viruses are considered but rejected.

III. This paper records the poliovirus isolation rate from faeces and pharyngeal secretions of household contacts of cases of poliomyelitis. These have been analysed to see if killed poliovaccine has any influence. 19 (44%) out of 43 with 1 or no doses of poliovaccine excreted virus in faeces against 9 (29%) out of 31 of those with 3 or 4 doses, a difference not taken to be significant. By contrast, significant differences in pharyngeal excretion were noted. Thus, 16 out of 43 who had 1 or no doses excreted virus as opposed to 3 out of 23 with 2 or more doses. *A. J. Beale*

Proctor, R., Melnick, J. L., Spence, L. & Downs, W. G. **Serological Epidemiology of Poliomyelitis on St. Vincent Island, Federation of West Indies.** *Amer. J. Trop. Med. & Hyg.* 1961, July, v. 10, No. 4, 579–82, 4 figs.

"Sera collected from 152 healthy young inhabitants of the island of St. Vincent (population, about 80,000) were assayed for antibody against all three types of poliovirus. Results showed that, in spite of the fact that the island has never had an outbreak of poliomyelitis, a significant part of the population develops antibodies to each of the three types of poliovirus by the age of 5 years, and this increases to involve 86 to 95% of the young adult population. Evidence indicates that at present St. Vincent is part of a Caribbean zone of poliomyelitis endemicity. But as clinical poliomyelitis is appearing with increasing frequency in other parts of this zone, vaccination of susceptible young children on St. Vincent should be considered in order to anticipate, and prevent, a shift to the epidemic situation."

Iwamura, N. **A Study on the Natural Defensive Mechanism for Poliomyelitis in the Non-Vaccinated Population.** *Yonago Acta Med.* 1961, Mar., v. 5, No. 1, 7–11, 3 figs.

"Non-epidemic factors of poliomyelitis in Yonago district, where no gross outbreak of this has ever occurred, have been studied.

"(1) The population in this district holds a high immunity rate. All children 7 years old and over had antibodies to all 3 types of poliovirus.

"(2) Both in positive rate of neutralizing antibody in serum and virus dissemination in nature Type III was leading."

Dick, G. W. A. & Dane, D. S. **The Control and Prevention of Outbreaks of Poliomyelitis.** *Med. Officer.* 1961, June 23, v. 105, No. 25, 369–71. [16 refs.]

The authors believe that in countries extensively vaccinated against poliomyelitis (with killed or attenuated vaccine) widespread epidemics will no longer occur. Localized outbreaks may occur, however, in poorly vaccinated areas as they have with diphtheria. The control of such outbreaks is considered in the light of recent findings that case-to-case transmission can be traced either directly or through contacts in 50% of the patients. First, it is recommended that child contacts of patients should be quarantined for 10 days (the exact duration of such quarantine should be the subject of investigation by titrating virus in faeces) but not adults since children are far more important in virus spread. Second, a leaflet should be distributed in the neighbourhood (the sensible leaflet prepared for use in Northern Ireland is given in an appendix). Third, immunization should be instituted. At present the authors recommend concentrated purified killed vaccine for close contacts and routine killed vaccine for other contacts. They favour this course because killed vaccine is still in routine use and because concentrated purified poliovaccine (Purivax, Merck, Sharp and Dohme) has been found by them to produce antibodies in monkeys in 4 days.

They make a somewhat inconclusive brief summary of the use of attenuated and killed vaccine in the face of an epidemic. They seem to be sceptical of the claims for the efficacy of living vaccine and they criticize

KNOWELDEN *et al.* [this *Bulletin*, 1961, v. 36, 1046] in regard to their recent re-examination of the Singapore outbreak in particular because no account was taken of the distribution of cases within the area. They point out that epidemic control is inefficient compared with adequate prophylactic immunization. They also point out that the system of outbreak control they have advocated when 1 case occurs can be graded much more flexibly to the needs of the situation by using killed vaccine than by blanketing the area with attenuated vaccine. It is difficult to decide whether or not to use attenuated vaccine when an outbreak occurs.

A. J. Beale

LAFONTAINE, A. & DE WEVER, A. Situation de l'épidémiologie poliomyélitique en Belgique après trois ans de vaccination par virus formolé. **[Epidemiological Situation Regarding Poliomyelitis in Belgium after Three Years of Vaccination with Formol Vaccine]** *Bruxelles-Méd.* 1961, July 23, v. v. 41, No. 30, 1067–79.

In the last 10 years 3 large outbreaks of poliomyelitis have occurred in Belgium, in 1952, 1955 and 1956. After 1956 the number of cases fell from 1,038 to 137 in 1959 but rose to 301 in 1960. The annual number of deaths varied between 12 and 85 during the period. The case fatality rate was 3·8, 8·6 and 4·0% in the epidemic years and from 5·7 to 20·5 in the years of low incidence. In 1959 and 1960 the majority of the patients were aged 1–4 years, 56·9 and 53·5% respectively, and the percentage of patients aged 21 years and over was 17·5 in 1959 and 19·9 in 1960. 128 of the 137 patients with poliomyelitis in 1959 had not been vaccinated and all of the 12 deaths were in the unvaccinated group. In 1960, 267 of the 301 had not been vaccinated and 18 deaths were recorded among the non-vaccinated and one among the 34 vaccinated patients.

The authors estimate that the protection obtained by vaccination lies between 75 and 97%. They discuss the future of vaccination in Belgium and suggest that the propaganda should be intensified, that preparation should be made for the administration of live virus, and that more study should be given to finding the best vaccination programme for young children. *W. J. Martin*

RUDNAI, O. & BARSY, G. **The Results of Salk Vaccination in Hungary as measured on the 1959 Poliomyelitis Epidemic.** *Acta Microbiologica.* Budapest. 1961, v. 8, No. 1, 103–13, 1 fig. [13 refs.]

In 1959 there was an extensive outbreak of poliomyelitis in Hungary. Approximately 80% of the population under 15 years were estimated to have had 2 or more doses (usually at least 3) of Salk vaccine before the 1959 outbreak. The majority of the children vaccinated in 1957 had received 2 doses of 0·2 ml. intracutaneously. They then received a third (0·5 ml.) dose intramuscularly. Groups born in 1957 or later had 1 ml. followed by 2 doses of 0·5 ml. Full details of numbers vaccinated and cases in vaccinated and unvaccinated groups are presented. The main comparison is made between thos with 3 or more doses and those with 2 or less. It concluded that the protection rate varied from about 5 to 70%. The case mortality rate was greater in un vaccinated than in vaccinated cases. 77% of the case in the triply vaccinated aged 3–8 years had had the 3r dose over 1 year earlier, suggesting that duration c immunity may be shortlived.

[The schedule of immunization used is known not t be optimally effective.] *A. J. Beale*

SERFLING, R. E., CORNELL, R. G. & SHERMAN, Ida I **The CDC Quota Sampling Technic with Result of 1959 Poliomyelitis Vaccination Survey** *Amer. J. Pub. Health.* 1960, Dec., v. 50, No. 1 1847–57, 3 figs.

Studies of poliomyelitis outbreaks in the United State of America in 1958 [MOLNER *et al.*, this *Bulletin*, 195 v. 34, 735; LANGMUIR, *ibid.*, 1960, v. 35, 1214] which i some areas revealed concentrations of paralytic attack of young children living in poor socio-economic cond tions, suggested the hypothesis that in such populatio groups there was a relatively low rate of artificia immunization compared with others in the same epidem area. No data in the sub-groups required (*i.e.* age, rac and social status) were available to test this hypothes and a survey technique for urban areas was planned t obtain such data.

The primary object was to estimate immunization rat for geographical sub-areas within each urban area, th sub-areas being defined by economic, racial and cultur characteristics. A pilot survey was carried out in Atlan in November 1958 and from May to June 1959 advic based on this pilot study, was given to the authoriti of 36 other cities. As an example the survey in Bosto in March 1959 is described in detail.

On the basis of 1950 census data on overcrowdin education and sanitation, the Boston census tracts we ranked in descending order or social status and divide into 3 socio-economic groups; the "upper" included th top 25% of ranks, the "lower" the bottom 25% an the "middle" the remaining 50%. From these thr groups two sub-groups were extracted and treate separately; they were a predominantly Negro central ar (Negro) and an old area of the city predominantly recent Italian culture (North End).

Out of 528 scheduled interviews (in the population about 800,000) 485 were completed giving informatio on the immunization state of 1,740 people. A table se out for each of the 5 sub-populations in 4 age grou (under 5, 5–14, 15–39 and 40 and over) the number persons who had had 0, 1, 2, 3 or 4 inoculations. It of interest to note that there were relatively more childr under 5 who had had 3 or more inoculations in Nor End (77%), which "to the casual observer . . . appea to be a typical slum", than in any other group ("upper 62%, "middle" 57%, "lower" 36% and Negro 30%

The results of some 26 other urban surveys are brie summarized. These followed the general plan used Boston with various local modifications. A modifi scheme is being developed with 2 pilot surveys for ru areas. Some of the results from the urban surveys ha

ed to public health action. The Boston survey won upport for mass immunization and some 30 strategically ocated clinics were established for this purpose. In St. Paul, Minnesota, clinics were set up in community centres and local libraries and in Dallas, Texas, similar clinics were established in church halls. In other towns ocal non-medical bodies, radio and television stations nd the local press were stimulated to give publicity and other forms of practical support to immunization campaigns.

The authors conclude that, before this acitvity which the surveys stimulated, the low immunization rates in young children in the lower socio-economic groups had been widespread. *E. A. Cheeseman*

MARINE, W. M., CHIN, T. D. Y., GRAVELLE, C. R. & SOERGEL, Marjorie E. **Effectiveness of Salk Vaccine in reducing Nonparalytic Poliomyelitis in a Large Urban Epidemic.** *New England J. of Med.* 1961, May 4, v. 264, No. 18, 903–7, 1 fig. [17 refs.]

The effectiveness of killed poliovaccine in preventing paralytic poliomyelitis has been demonstrated many times. Its effectiveness against "nonparalytic poliomyelitis" has not been established, presumably because this syndrome is not often due to poliovirus. In the 1959 poliomyelitis outbreak in Kansas there were 118 paralytic and 92 non-paralytic cases and most were due to type 1 poliovirus. The effectiveness of 3 doses of vaccine was found to be 76·8% for paralytic and 73·5% for non-paralytic disease. The cases were concentrated mostly in the Negro population up to 4 years old, which was the least well vaccinated. *A. J. Beale*

PAN AMERICAN HEALTH ORGANIZATION & WORLD HEALTH ORGANIZATION, with the co-operation of SISTER ELIZABETH KENNY FOUNDATION. **Second International Conference on Live Poliovirus Vaccines (Washington, D.C., 6–10 June 1960). Papers presented and Discussions held.** pp. xv + 634, illustrated. [Numerous refs.] Scientific Publication No. 50. 1960. Wash. 6: Pan American Sanitary Bureau, Regional Office of the W.H.O., 1501, New Hampshire Avenue, N.W., D.C., U.S.A. [$5.00.]

[Much of the information presented at this conference has since been published in scientific journals.]

After an opening session, at which H. KOPROWSKI (pp. 5–11) celebrated the "tin" anniversary of attenuated poliovaccine development, there was a prolonged discussion on laboratory evidence of attenuation and safety. At this session B. H. SWEET and M. R. HILLEMAN (pp. 79–85) reported the discovery of vacuolating virus [see this *Bulletin*, 1961, v. 36, 506], a virus present in many cultures of normal rhesus monkey kidney, which produces cytopathic effects on vervet monkey kidney cells (*Cercopithecus aethiops*). R. KIRSCHSTEIN, G. BORMAN, S. BARON, R. FRIEDMAN, R. MURRAY and G. HOTTLE (pp. 90–97) described central nervous system lesions after intramuscular inoculation of virus. The Lederle and Koprowski type 1 strains in large dosage caused paralysis whereas Sabin's strains only caused histological lesions confined to the lumbar cord in animals inoculated in the big muscles. H. PETTE, H. LENNARTZ, G. MAASS, L. VALENCIANO and K. MANNWEILER (pp. 68–79) also confirmed earlier reports that the Sabin strains were less neurovirulent than the Cox strains. Several speakers, J. L. MELNICK, V. J. CABASSO, H. L. HODES, H. KOPROWSKI, P. R. LÉPINE and F. C. ROBBINS (in discussion) reported work on *in vitro* markers and their correlation with neurovirulence. The d, t and MS markers, although useful for "finger-printing" the attenuated strains, were not universally correlated with neurovirulence. New markers were also described; that of MCBRIDE [this *Bulletin*, 1959, v. 34, 833] for intratypic antigenic specificity and the elution pattern of attenuated viruses differed from that of virulent viruses. R. DULBECCO felt that the adsorption marker might be most closely related to neurovirulence since it was probably a surface property. A. B. SABIN suggested that capacity to adsorb to central nervous system [CNS] receptor substance [Sabin, 1st International Conference on Live Poliovirus Vaccines, this *Bulletin*, 1961, v. 36, 276] might be important. S. BARON described an inhibitor in the CNS which was active against attenuated but not to the same extent against virulent viruses. SABIN discussed "cold mutants" of poliovirus (pp. 101–108) which did not seem to offer advantages over existing strains.

Field evidence of safety was next discussed with emphasis on 2 aspects: stability on human passage and spread. It is clear from papers given at the session on genetic markers that virus on passage in the human gut tends to change its genetic character however measured, by virulence, by CNS or intramuscular route or by *in vitro* markers, although D. M. HORSTMANN, J. R. PAUL, E. P. ISACSON and J. C. NIEDERMAN (pp. 113–120) reported a trial in which little change in *in vitro* markers occurred. J. P. FOX, D. R. LE BLANC, H. M. GELFAND, D. J. CLEMMER and L. POTASH (pp. 144–155) and A. C. KIMBALL, R. N. BARR, H. BAUER, H. KLEINMAN, E. A. JOHNSON and M. COONEY (pp. 161–174) studied spread and found that even in households there was less spread with the Sabin type 3 strain studied than with virulent ones. It was thought, therefore, that fears about reversion to virulence were needless and hopes that involuntary vaccination would take care of those unwilling to be vaccinated likely to be disappointed. J. R. PAUL, D. M. HORSTMANN, J. T. RIORDAN, E. M. OPTON and R. H. GREEN (pp. 174–184) found that intrafamily spread with Lederle type 3 virus was quite extensive. They also found that type 3 in a trivalent mixture interfered with types 1 and 2. K. A. PREM, J. W. FERGUS, J. E. MATHERS and J. L. MCKELVEY (pp. 207–227) gave vaccine to pregnant women and young infants without untoward effects. No evidence of development of immunological tolerance or foetal damage was found although few women at a vulnerable stage of pregnancy were given the vaccine.

Two other papers [M. E. FLIPSE, G. M. ERICKSON, W. R. HOFFERT, M. M. SIGEL, N. J. SCHNEIDER, L. B. CLAYTON, A. W. MENZIN, R. E. MARKUSH, F. HOWELL, Jr., M. I. CROSSLEY, T. E. CATO, A. V. HARDY and F. J. EVANS (pp. 435–444); G. M. ERICKSON, M. E. FLIPSE, A. W. MENZIN, L. B. CLAYTON, R. E. MARKUSH and A. V. HARDY (pp. 445–456)] raised questions of safety. During the campaign with Lederle vaccine in Dade County there were 7

cases of paralytic poliomyelitis which was less than the 5-year mean. The disturbing feature about these cases was that 5 occurred within 2 weeks of taking the vaccine and 5 were in adults. F. M. BURNET (p. 460) remarked that if attenuated polioviruses were to cause cases this was just the sort of episode to be expected.

S. A. PLOTKIN, A. LEBRUN, G. COURTOIS and H. KOPROWSKI (pp. 466–473) gave an account of the trial in Leopoldville of Koprowski's type 1 strain. This was judged to be about 67% effective. 39 cases occurred in vaccinated individuals, 5 within 1 month (this is less than the 7–8 cases expected by chance). The genetic markers of strains isolated from cases in the vaccinated were presented from which it was concluded that these strains were wild and not reversions to virulence of the vaccine strain. R. DULBECCO felt, however, that the possibility had not been excluded by the marker tests that had been performed. The use of the vaccine in large scale campaigns was presented by M. P. CHUMAKOV *et al.* (pp. 413–428), V. M. ZHDANOV (pp. 576–588) and A. A. SMORODINTSEV, I. A. DROBYSHEVSKAYA, N. P. BULYCHEV, O. M. CHALKINA, G. M. GROISMAN, V. I. ILYENKO, R. A. KANTOROVICH, L. M. KURNOSOVA, K. G. VASILIEV, V. I. VOTIAKOV and G. P. ZHILOVA (pp. 482–501) from Russia and V. SKOVRÁNEK from Czechoslovakia (pp. 507–521).

M. R. ALVAREZ, M. E. BUSTAMENTE and R. A. ALBA (pp. 386–409) reported the trial of Sabin's vaccine in Mexico in which he used a single dose of trivalent vaccine in a mass blitzkrieg in an effort to break the chain of infection. This method seemed most effective. Reports from Nicaragua (J. J. ALCOCER, M. D. R. ARMIJO and M. MARTINS DA SILVA (pp. 547–560)) and Costa Rica (J. NÚÑEZ, O. VARGAS-MÉNDEZ, E. C. GUEVARA, J. M. QUIRCE, J. A. MONTOYA, H. DOANY and M. MARTINS DA SILVA (pp. 561–568); J. M. QUIRCE, J. NÚÑEZ, E. C. GUEVARA, J. A. MONTOYA, H. DOANY and A. SHELOKOV (pp. 569–573)) stressed the advantage of a trivalent mixture from the point of view of the public health worker. Both these countries experienced high attack rates of poliomyelitis after the mass campaign with rates, as A. D. LANGMUIR noted in discussion, similar to those found in the U.S.A. in minority poorly vaccinated groups in otherwise well vaccinated populations. In Nicaragua there were substantial numbers of cases in vaccinated individuals.

At the final session of the conference a masterly short summary was presented by A. M. M.-PAYNE (pp. 600–603). This should be read by anyone wishing to get a "thumbnail" synopsis of the conference.

A. J. Beale

FOX, J. P. **Live Virus Immunization. A Consideration of Basic Principles and Possible Pitfalls.** *Arch. Environment. Health.* Chicago. 1961, May, v. 2, No. 5, 490–98. [37 refs.]

This is a long, general review of living virus vaccines as impartial as possible in one with a declared bias in favour. First natural infection is considered to give life-long immunity to the disease and also to infection. Killed vaccines suffer from several disadvantages: large quantities of antigen are required and this is difficult to achieve without either causing direct toxicity or toxicity from impurities. At least 2 injections are required and usually more. Immunity to disease may be achieved but not to infection. Safety requires complete killing of the agent. Living vaccines, by contrast, ideally should mimic natural infection and produce life-long immunity without causing disease. There are several problems in isolating and attenuating strains of disease-producing viruses. A proper system of maintaining attenuation is required, in view of the mutability of viruses, and of producing the attenuated vaccine free from extraneous contaminating viruses. The problems of licensing involving standards of safety and effectiveness under routine use as well as under the special conditions of trials are briefly considered.

[This is an interesting account.] *A. J. Beale*

MARÓCZI, J., RÉTHY, L., KELEMEN, G. & PÁCSA, S. **Trivalent Oral Poliomyelitis Vaccine and Combined Diphtheria-Tetanus-Pertussis Immunisation.** [Correspondence.] *Lancet.* 1961, July 15, 159–60.

Infants aged 3 to 9 months were immunized with 2 doses of diphtheria, pertussis and tetanus antigens and half of them also received 2 oral doses of trivalent attenuated poliovaccine. Immunity was assessed 56 days after the 1st dose or 28 days after the 2nd dose. The simultaneous administration of the attenuated polio vaccine and triple antigen had no effect on the diphtheria tetanus or poliovirus antibody responses. Unfortunately no results are given for the pertussis component, nor are there any details about the dose of the antigens, which is particularly unfortunate for the poliovaccine. The results were not good; whereas 46 out of 46 and 43 out of 43 infants were immune to diphtheria and tetanus after vaccination, the figures for poliomyelitis were 13 out of 31 for type 1, 23 out of 30 for type 2 and 19 out of 30 for type 3. *A. J. Beale*

LENNARTZ, H. & VALENCIANO, L. Virologische Untersuchungen zur oralen Schutszimpfung gegen Poliomyelitis in Westberlin 1960. **[Virological Studies during Oral Polio-Vaccination in West Berlin in 1960]** *Deut. med. Woch.* 1961, Aug. 11, v. 86, No. 32, 1497–503, 1535, 3 figs. [13 refs.]

The English summary appended to the paper is as follows:—

"Antibody formation and virus excretion after oral administration of the trivalent polio vaccine (Lederle 1960) in 139 children in a children's home in Berlin are described. An effective antibody response was obtained against types I and III. As for virus multiplication, it was most marked for type III with type I following. Type II was least multiplied, which explains the poor effectiveness of antibody response against it. The importance of contact infection was underlined by the fact that all unvaccinated children of this home without antibodies against type I or III were infected with these two types by contact with the vaccinated children. Type I strains of healthy excretors at the children's home proved to be stable with regard to temperature and M[illegible] marker determinations, while four of the investigated

type-III strains showed no correspondence with the original material. Most of the strains isolated from vaccinated children with the disease and the diseased contacts were avirulent by in vitro and in vivo tests. In addition, the wild strains isolated before the vaccination programme were found to be naturally attenuated. Serological differentiation allowed the definition of some of the wild viruses from the so-called vaccination cases. Most of the strains of these patients however were found to be homologous with the vaccine strains. It is pointed out that it is difficult after the oral administration of a trivalent vaccine to bring proof of the causal relationship between contracted polio and the demonstrated type of virus. However, in some cases supposedly due to the vaccination a relationship must with great probability be assumed with the isolated virus which was shown to be one of the vaccine strains. Since there is little evidence of an increase in virulence in the material examined, the conclusion seems justified that even attenuated polio viruses can in exceptional cases cause the full-blown picture of poliomyelitis."

BUSER, F. & SCHAR, M. **Poliomyelitis Vaccination with Poliovirus.** *Amer. J. Dis. Children.* 1961, May, v. 101, No. 5, 568–74, 7 figs. [19 refs.]

A trial of Koprowski types 1 and 3 attenuated polio-vaccine in Bern, Switzerland, is reported. 256 people were given type 1 and 79 of them type 3. The results were excellent, only 2% and 5% for types 1 and 3 remained without antibodies and 74% of a sample of triple negatives had titres of more than 1 in 200 to type 1 and 64% to type 3. 10^6 TCD50 of type 1 and 10^5 TCD50 of type 3 constituted a dose. Type 1 contact infections occurred in 25% of the child-family contacts in one-third of the families. 2 of the strains isolated from contacts were not neurovirulent for monkeys by the intracerebral route. The antibodies produced by living vaccine persisted substantially unchanged for 1½ to 2 years. *A. J. Beale*

ŠKOVRÁNEK, V. **Nation-Wide Vaccination with Live Poliovirus Vaccine in Czechoslovakia in 1960.** *Rev. Czechoslovak Med.* Prague. 1961, v. 7, No. 1, 1–11, 2 figs. [18 refs.] [With an appendix Instructions for the Preparation, Organization, Implementation and Evaluation of Protective Vaccination against Poliomyelitis with the Sabin Vaccine issued by Ministry of Health, 11–19.]

An account of the campaign with living vaccine in Czechoslovakia is presented. In an appendix, detailed instructions on the campaign and follow-up sent out by the Ministry of Health are published. The vaccine was given in 2 doses: first, type 1 starting 28 March, 1960 and completed in 10 days, and then on 2 May types 2 and 3 together. This method was dictated by pressure of time but it is still believed that the types should be given singly for optimal results. $10^5 - 10^{5\cdot3}$ TCD50 of all 3 types of virus were given. When the 3 types are given together in this dosage a fall in type 1 serological response from 96% (when given alone) to 60% is said to occur. With the schedule actually employed for the campaign the results for types 1 and 2 were said to be as good as when given individually, but the type 3 response was slightly worse. The campaign was successfully carried out. More than 3½ million people were vaccinated, 94% of the age-group 2 months to 14 years with type 1 and 93% with types 2 and 3. The vaccine did not cause any ill effects and was thought to be effective in preventing poliomyelitis in that after the completion of the campaign there were only 3 cases of confirmed paralytic poliomyelitis in the whole of Czechoslovakia, and 2 of these were imported.

[This is an interesting and informative account of the organization but not a scientific appraisal of the results.]

A. J. Beale

ŠKOVRÁNEK, V. & ŽÁCEK, K. **Oral Poliovirus Vaccine (Sabin) in Czechoslovakia. Effectiveness of Nation-Wide Use in 1960.** *J. Amer. Med. Ass.* 1961, May 13, v. 176, No. 6, 524–6, 1 fig.

This is a shorter version of the paper by ŠKOVRÁNEK, reviewed above.

LEPOW, Martha L., WARREN, R. J., GRAY, N., INGRAM, Virginia G. & ROBBINS, F. C. **Effect of Sabin Type 1 Poliomyelitis Vaccine Administered by Mouth to Newborn Infants.** *New England J. of Med.* 1961, May 25, v. 264, No. 21, 1071–8, 5 figs. [26 refs.]

It would be advantageous to be able to immunize infants against poliomyelitis early in life while the mother is still under medical care. This would ensure protection from the earliest moment. Unfortunately, infants respond poorly to many antigens, including killed poliovaccine.

In this trial 144 newborn infants were fed $10^{7\cdot5}$ TCD50 of Sabin type 1 virus, and 70 infants aged 10–16 weeks were fed 10^6 TCD50 of the same virus. Many of the infants were Negroes and almost all were in the lower socio-economic group. Passive antibody levels were high. It was found that passively acquired antibodies inhibited virus excretion. Thus, in the newborn infants with maternal antibody titres of 128 or less, 73% excreted virus for more than 1 week, in those with titres of 256 or 512 62% did so, and in those with titres of 1024 or more only 35% did so. The total virus excretion rates were higher, but the proportions making an antibody response confirmed the trend, being 83%, 44% and 16%, respectively. The criterion of response was a titre 8-fold higher than would be expected on the assumption of a decline of maternal antibodies with a half life of 45 days. This, as the authors note, is more stringent than the standard adopted by earlier workers. The overall antibody production rate was 50% for newborn infants and over 90% for those aged 3 months. It was found that the small numbers of breast-fed infants with maternal titres over 128 excreted virus significantly less often than bottle-fed infants with the same titre. Wild enteroviruses were not present during the study, and spread of poliovirus was not detected. *A. J. Beale*

PORTER, E. R. & WEHR, R. E. **Oral Poliomyelitis Vaccine Program in Cincinnati.** *Pub. Health Rep.* Wash. 1961, May, v. 76, No. 5, 369–74, 2 figs.

The organization of the Cincinnati attenuated polio-vaccine programme carried out in the spring of 1960 is described. During two 1-week periods 67,634 children aged 3 months to 5 years and 111,127 schoolchildren were given type 1 Sabin vaccine. 4 weeks later, 91% of the pre-school children returned for type 3 and 83% for type 2 another 4 weeks after the type 3. In one area [not clearly defined] 50,726 children 3 months to 5 years old were vaccinated [presumably with type 1 virus] representing 73·4% of the age-group. The business community helped the campaign particularly by donating advertising and transport. Voluntary helpers did the record-keeping. Doctors helped both by opening their offices for free vaccination and attending clinics. Publicity stressed that the vaccine was given with a spoon not a needle. Special efforts were required in parts of the city responding least well, to get the children to the clinics. In 1960 there were no proven cases of poliomyelitis in Cincinnati residents. *A. J. Beale*

FLIPSE, M. E., ERICKSON, G. M., HOFFERT, W. R., SIGEL, M. M., SCHNEIDER, N. J., CLAYTON, L. B., MENZIN, A. W., MARKUSH, R. E., HOWELL, F., Jr., CROSSLEY, M. I., CATO, T. E., HARDY, A. V. & EVANS, F. J. Informe preliminar sobre un estudio de campo en gran escala con vacuna Cox-Lederle de virus poliomielítico atenuado, administrada por vía oral, en el Condado de Dade (Miami), Florida. **[Preliminary Report on a Large-Scale Field Study with Cox-Lederle Attenuated Poliomyelitis Virus Vaccine Administered Orally in Dade County, Florida]** *Bol. Oficina Sanitaria Panamericana.* 1961, Feb., v. 50, No. 2, 120–30.

As a result of unsatisfactory protection conferred by Salk-type poliomyelitis vaccine in Dade County, Florida, and an increasing apathy of the public towards poliomyelitis vaccination, it was decided to carry out large-scale vaccination with live vaccine offered to all residents of the area under 40 years of age. Vaccination was carried out in February, March and April by a single oral dose of 2 cc. of Lederle-Cox vaccine containing $10^{6\cdot1}$ TCD50 of type 1 (SM strain) and type 3 (Fox), and $10^{6\cdot3}$ TCD50 of type 2 (MEF 1 strain) poliomyelitis virus. 411,279 persons were vaccinated in all. The antibody response was investigated in sera from 594 persons of all ages who lacked antibody to 1 or more types of poliomyelitis virus before administration of the live vaccine, 302 of whom had received Salk vaccine. 538 (91%) developed neutralizing antibody in titres of 4 or higher after administration of the live vaccine; 42 of 56 (75%) failures were in respect of type 2. Among the 885 individual negative results to individual types before live vaccination, 817 positive results in a titre of 4 or higher and 726 in a titre of 16 or higher were obtained after vaccination; the conversion rate was 95% (285 of 300) for type 1, 78% (152 of 194) for type 2, and 97% (380 of 391) for type 3. *D. J. Bauer*

NUÑEZ, J.; VARGAS-MENDEZ, O.; GUEVARA, E. C. QUIRCE, J. M.; MONTOYA, J. A.; DOANY, H. MARTINS DA SILVA, M.; SHELOKOV, A. La vacu nación con virus poliomielítico atenuado en Cost Rica. Segundo informe sobre la marcha del estudic Sección I. Programa de vacunación. **[Vaccinatio with Attenuated Poliomyelitis Virus in Cost Rica. Second Report on the Progress of th Study. I. Vaccination Programme]** [NUÑEZ VARGAS-MENDEZ, GUEVARA, QUIRCE, MONTOYA DOANY & MARTINS DA SILVA]. *Bol. Oficina Sani taria Panamericana.* 1961, Feb., v. 50, No. 2, 109 15, 3 figs. Sección II. Programa de vigilancia **[II. Follow-up Programme** [QUIRCE, NUÑEZ GUEVARA, MONTOYA, DOANY & SHELOKOV]. *Ibid.* 115–19.

I. The annual number of cases of poliomyelitis i Costa Rica has fallen from 1,081 in 1954 to 31 in th first quarter of 1960, most cases occurring in chlidre below 5 years of age. Vaccination of children up to th age of 11 with monovalent live vaccine was begun i 1959, but this was later changed to trivalent vaccine as about 15% of the children failed to complete th course. 271,631 children were vaccinated among a tota of 382,905 in the age-group (71%). In the examinatio of sera from 726 children receiving the course of mono valent vaccine the percentage conversions from the sero negative state were 82, 37 and 92 for types 1, 2 and respectively, and similar results were obtained with th use of trivalent vaccine.

II. 59 cases of paralytic poliomyelitis were observed i Costa Rica in 1959–60 after the beginning of the vaccina tion campaign, 14 of which occurred in children who ha been vaccinated. 1 case occurred 10 days after vaccina tion and the remainder after at least 7 weeks. Th attack rate during the period concerned was 128 pe 100,000 for unvaccinated and 14 per 100,000 for vac cinated persons. *D. J. Bauer*

DROZDOV, S. G. & SHIRMAN, G. A., with the technica assistance of T. V. KNYAZEVA. **Interaction o Viruses in the Intestinal Tract of Man. I. Inte ference between Wild and Vaccine Polioviru Strains.** *Acta Virologica.* Prague. 1961, July v. 5, No. 4, 210–19, 8 figs. [12 refs.]

Interference between wild type 2 poliovirus and type Sabin attenuated poliovaccine was studied. 46 childre not excreting any virus before vaccination were succes fully immunized with a dose of about 10^5 TCD50 of th Sabin vaccine. 17 children were excreting type 2 polio virus and in 5 this virus prevented growth of the type vaccine and in these the titres of type 2 were high befo vaccination. In 12 children the vaccine strain was estab lished and the wild strain eliminated in 2–6 days. I these children the pre-vaccination titres of type 2 we low. 3 children failed to be immunized despite lac of a virus in the pre-vaccination faecal sample becaus they became infected with the wild type 2 instead of th vaccine strain. The authors believe that wild viruses ma be a serious factor in marring the success of attenuate poliovaccination programmes unless they are done in th

winter when circulation of intestinal viruses is less and, preferably, on a mass scale, so that nature helps the spread of the attenuated strains. *A. J. Beale*

BAIN, H. W., MCLEAN, D. M. & WALKER, Selma J. **Epidemic Pleurodynia (Bornholm Disease) due to Coxsackie B-5 Virus. The Interrelationship of Pleurodynia, Benign Pericarditis and Aseptic Meningitis.** *Pediatrics.* Springfield, Ill. 1961, June, v. 27, No. 6, 889–903. [39 refs.]

This is a most interesting review of Bornholm disease and an account of an epidemic of Coxsackie B5 infection in Southern Ontario in 1958, manifested by pleurodynia, benign pericarditis and abacterial meningitis. There is a particularly valuable discussion of the 7 cases of benign pericarditis. A patient in this outbreak who subsequently developed constrictive pericarditis is referred to briefly. *A. J. Beale*

WILSEN, A. A. J.; PEISACH, H.; HOWARTH, W. H.; NAUDÉ, W. du T.; BECKER, W B.; MALHERBE, H. H.; HARWIN, R. **A Closed Epidemic of Acute Aseptic Meningitis caused by ECHO Virus Type 4. Part I. Epidemiological and Clinical Studies** [WILSEN, PEISACH & HOWARTH]. *South African Med. J.* 1961, Apr. 22, v. 35, No. 16, 330–33, 3 figs. [10 refs.] **Part II. Laboratory Studies** [HOWARTH, NAUDÉ, BECKER, MALHERBE & HARWIN]. *Ibid.*, 333–5.

These 2 papers describe an outbreak of ECHO 4 meningitis in the Schools for the Deaf and Blind at Worcester, Cape Province, South Africa. 200 pupils and 4 staff out of 418 and 64, respectively, fell ill. The main symptoms were: headache, 100%; pyrexia, 91%; giddiness, 84%; nausea and vomiting, 75%; rigors, 41%; neck stiffness, 32%; conjunctivitis, 21%. ECHO virus type 4 was isolated in vervet monkey kidney cultures from 56% of the 41 CSF samples, 22% of the 32 throat swabs and 6% of the 33 rectal swabs. 7 out of 10 paired sera showed the development of some antibody evidenced by partial inhibition of ECHO 4 virus. [ECHO 4 virus is unsatisfactory for conventional neutralization tests (see ITOH and MELNICK, this *Bulletin*, 1958, v. 33, 290).] *A. J. Beale*

WALLACE, H. L., EDGE, W. E. B. & CLYDE, J. H. **Acute Aseptic Meningitis in Children.** *South African Med. J.* 1961, June 3, v. 35, No. 22, 450–53, 1 fig. [11 refs.]

During the years 1953–60 inclusive, 161 children with acute meningitis (cases of tuberculous meningitis were excluded) were admitted to Addington Children's Hospital, Durban. Of these cases 73 were of proved or presumed bacterial origin, while 88 (55%) were of the abacterial variety, presumably viral in origin.

The annual incidence varied similarly but not in close correspondence to cases of poliomyelitis notified, while the seasonal incidence was much higher in the summer months (73% in November to March) as with poliomyelitis, and contrasting with the predilection of septic meningitis for the winter months.

Presenting symptoms included fever, vomiting and headache in 66% or more of the patients. Convulsions occurred in 10%. Neck stiffness was the most frequent sign, occurring in 51%.

As regards changes in the cerebrospinal fluid the authors point out that at the first lumbar puncture polymorphonuclear leucocytes are found as often as lymphocytes, and that while the total white cell count is usually under 200 per cmm. in 15% it was higher, overlapping the range found in septic meningitis. Proteins exceeded 40 mgm. per 100 ml. in only 30%. Chloride estimation was of no value in diagnosis, and normal sugar values were found in too many of the septic cases to be a reliable indicator.

Virus studies were carried out in 45 of the 88 patients. In only 25% of these was confirmation of viral aetiology obtained. The infecting agents included mumps, poliomyelitis, Coxsackie, and ECHO viruses.

No specific treatment was available or found necessary, the condition being benign, and clinical recovery occurring quickly, though cells may persist in the cerebrospinal fluid for many weeks. The authors emphasize the value of clinical assessment in the diagnosis, particularly their observation that the child is not as ill as would be expected. *A. C. E. Cole*

KARZON, D. T., ECKERT, G. L., BARRON, A. L., HAYNER, N. S. & WINKELSTEIN, W., Jr. **Aseptic Meningitis Epidemic due to ECHO 4 Virus.** *Amer. J. Dis. Children.* 1961, May, v. 101, No. 5, 610–22, 3 figs. [28 refs.]

In Buffalo, N.Y. in 1956, ECHO virus type 4 was isolated from 66% of 124 cases of aseptic meningitis; in 32% of the virus-positive cases virus was recovered from the cerebrospinal fluid.

The virus isolation rate was greater in children under 15 years of age (81%–90% positive) than in adults (50%). Virus excretion was mainly faecal, lasting from 1–3 weeks after the onset of illness; throat swabs were positive only in the first week. In general the period of excretion was longer in children than in adults.

The attack rate of aseptic meningitis associated with ECHO virus type 4 infection was 88·4 per 100,000 in the predominantly Negro areas of the city first affected. The subsequent attack rate in the white population of adjoining areas was 2·6 per 100,000.

In 71 households with a case of ECHO virus type 4 meningitis a secondary attack rate of 11% was found, with an apparent incubation period of 5–8 days. In 320 members of 58 affected households, 82 had a recent history of minor febrile illness; ECHO virus type 4 was isolated from 36 people in these families, mainly children under 5 years of age, and in 11 of these instances there was no history of recent illness.

In 3 children with virus-positive abacterial meningitis a maculopapular rash occurred. However, no cases of febrile exanthematous disease alone seem to have occurred during this outbreak. *Derek Hobson*

TURCHETTI, A. **Virus Hepatitis in Eastern Sicily.** *Panminerva Med.* Turin. 1961, Jan., v. 3, No. 1, 9–13. [Translation from *Minerva Med.* 1960, v. 51, 1862.]

This is an important contribution to the study of infectious hepatitis. It deals with a region (Eastern Sicily) in which the disease is exceptionally severe and where the opportunity exists for assessing the various causative factors. In over 100 patients admitted to the Institute of Pathology, Palermo, the diagnosis was verified by serological tests and often by biopsies, and epidemiological enquiries were also made in the vicinity over a period of 3 years. The mortality rate during jaundice was very high—11% among in-patients and 3% among out-patients. The post-jaundice course of the disease was traced out. A long irregular course of ill-health was common, marked by ascites, haemorrhage, and ultimately coma; and biopsies in these cases showed that the relapsing parenchymal damage due to the virus combined with the mesenchymal cellular reaction gradually evolved into hepatic cirrhosis. An enlarged hardened liver and an enlarged spleen (not now, in Sicily, caused by malaria) were often found in such cases. A special study of 3 families, in which most of the members fell ill with acute hepatitis at intervals of about 6 months, revealed that one member of each family had chronic cirrhogenous hepatitis and was probably the source of intermittent infection.

Evidence is given for the belief that the severity of hepatitis in this region is associated with 3 factors—relatively advanced age of the patients (45–65 or older), early return to hard work in field or factory after an acute episode, and the poor (low-protein) diet of the inhabitants. *H. Stanley Banks*

SEAL, S. C., MUKHERJI, B. & BOSE, S. B. **Infectious Hepatitis in the Gomoh Railway Colony (Bihar), 1959.** *Indian J. Pub. Health.* 1960, Apr., v. 4, No. 2, 67–83, 2 charts & 1 map on 2 folding pls.

A long detailed account is given of the water supplies and sanitation (or lack of it) available in a railway colony (population 11,064) in which 165 cases of infectious hepatitis with jaundice were reported in January to May 1959, the main incidence being in the running and locomotive engine staff. Owing to shortage of the filtered, chlorinated water supply, these men frequently drank water from the loco-tank, a ground reservoir of water intended for engines, fire-fighting, and carriage washing. This water came from a small stream which passed through a nullah that was subject to much faecal contamination. This was probably the source of the outbreak. The number of females and children affected was very small and they were nearly all secondary cases. [This population had probably a fairly high degree of immunity and the epidemic appeared to be caused by the drinking of the most grossly contaminated of the available water supplies.] *H. Stanley Banks*

HILLIS, W. D. **An Outbreak of Infectious Hepatitis among Chimpanzee Handlers at a United States Air Force Base.** *Amer. J. Hyg.* 1961, May, v. 73, No. 3, 316–28. [23 refs.]

No animal other than man has so far been found to be susceptible to the virus or viruses of infectious hepatitis. This report of an outbreak of the disease apparently caused by handling young chimpanzees during the first few weeks or months after their capture is therefore of great interest. Among the 21 Air Force personnel who had such contacts 9 clinical and 2 sub-clinical (positive serum glutaminase test) attacks of hepatitis were recorded —an attack rate of over 50%. A control group of 88 personnel of the Base having no history of contact with chimpanzees showed no clinical or sub-clinical hepatitis. Further, some 75 persons who experienced repeated contacts with older chimpanzees in the established animal colony developed no known hepatitis during the period of contact, and serum transaminase (glutamic-oxeloacetic) determinations were all normal. Epidemiological inquiries failed to reveal any source of infection other than the young chimpanzees of 1 to 1·5 years of age, all recently captured. Bearing on this it was discovered that many, if not all, of these chimpanzees had received from their African vendors an intra-peritoneal injection of pooled human blood in order to protect them against human diseases.

It would seem from this well-documented report that young chimpanzees might serve as transient carriers of human hepatitis viruses under such circumstances.

H. Stanley Banks

COMBESCOT DE MARSAGUET, G. & THOMAS, J. Au sujet de cinq cas de guérison de coma hépatique d'origine virale au cours de la grossesse. [**Recovery of 5 Patients from Hepatic Coma of Viral Origin during Pregnancy**] *Méd. Trop.* Marseilles. 1961 Jan.–Feb., v. 21, No. 1, 45–58. [43 refs.]

The authors observed 50 cases of viral hepatitis in pregnant African women in the [former French] Congo of whom 35 had benign infections and 15 had hepatic coma; of the latter 10 died and 5 recovered. This is a remarkably good result in a disease that is almost invariably fatal to both mother and child unless it is vigorously treated. The subject is discussed in terms of clinical findings, aetiology and pathogenesis, prognosis, and treatment. Stress is laid upon the efficacy of modern treatment of hepatic coma, especially the use of cortisone, glutamic acid, and aureomycin. Indeed the loss of 9 of the 10 patients who died is attributed largely to the inadequate treatment given because the means were not available. Cortisone is believed to be the essential element in the treatment, and dosage of glutamic acid should not be less than 10 gm. daily since its suppression or non-administration in 41 cases of hepatic coma of all kinds was observed to lead to a check in the progress of the case.

The various therapeutic agents used were: cortisone 100 mgm. daily injected during the comatose period and then deltacortisone by mouth in diminishing dosage from 25 to 15 mgm. daily for 3 weeks; glutamic acid in the form of magnesium glutamate intravenously 10–15 gm daily during the coma and then oral administration for a few days; aureomycin intravenously 200–400 mgm daily; gamma globulin injected at 2-day intervals; liver extract also administered in the intravenous drip, and methionine during the coma; vitamins B1, B2, pp, B12, C

and K also injected at intervals. In spite of restlessness and convulsive seizures, tranquillizers were little used.
H. Stanley Banks

TRÜB, C. L. P., ALTHAUS, H. & POSCH, J. Abakterielle Gastroenteritis-Epidemie und Trockenwetterperiode im Ruhrwasserversorgungsgebiet Spätherbst und Winter 1959/1960 im Lande Nordrhein-Westfalen. [**An Epidemic of Abacterial Gastro-Enteritis in the Water Conservation Area of the Ruhr, in the Autumn and Winter of 1959–60**] *Arch. f. Hyg. u. Bakt.* 1961, June, v. 145, No. 4, 302–20. [Numerous refs.]

The introduction to this lengthy paper contains much reference to the literature on epidemics of non-bacterial gastro-enteritis which have been regarded as or shown to be of viral origin.

The epidemic here described in considerable detail occurred in the Ruhr district. It began with sporadic cases of gastro-enteritis in the autumn of 1959 and rose to a peak in December. The number of ascertained cases in the areas of Essen and Mülheim during the epidemic was at least 80,000 and it is estimated that between 5% and 10% of the population were affected in different communities.

There is an account of the clinical types of the disease which affected all ages but particularly infants and small children. Exhaustive investigations of the faeces yielded no definite evidence of bacterial or viral causation.

From June on through the following months of 1959 the weather had been exceptionally dry and water supplies had become very depleted. Examination of these supplies showed that the waters had deteriorated in quality on the evidence of their greatly increased chloride, organic matter, nitrate, nitrite and ammonia content. In addition it was determined that the concentration of detergents had also increased considerably.

The findings of all these tests are described in great detail. The problem of the possible relation between the gastro-enteritis which is probably caused by enteroviruses and the changes in the water supply is discussed at length. It is possible that the increased concentrations of salts and of surface-active substances may have enhanced the pathogenic effect of enteroviruses also present in the water.
M. E. Delafield

KOKERNOT, R. H., SMITHBURN, K. C., GÂNDARA, A. F., MC'INTOSH, B. M. & HEYMANN, C. S. Provas de neutralização com soros de indivíduos residentes em Moçambique contra determinados vírus isolados em África transmitidos por artrópodes. [**Neutralization Tests for Arborviruses in Residents of Mozambique**] *Anais Inst. Med. Trop.* Lisbon. 1960, Jan.–June, v. 17, Nos. 1/2, 201–30, 1 fig. [28 refs.] English summary.

Human sera were collected in 1957 from residents in 29 widely separated places in Mozambique from both adults and children (aged under 15 years). They were tested in mouse neutralization tests for antibody to 4 group A and 5 group B arthropod-borne viruses, to Bunyamwera and to 5 ungrouped arthropod-borne viruses all of which have been isolated in Africa.

The results were as follows:

	Children %	Adults %	Number monotypic
Chikungunya	4	37·5	150 of 191
Semliki Forest	3	7	15 of 44
Sindbis	5	9	48 of 64
Middelburg	0·7	2	0
Wesselsbron	10	21	103 of 138
Spondweni	1	6·5	15 of 35
H 336	2·5	6	14 of 39
Uganda S	1	5	0
Zika	2	6	0
Bunyamwera	16	31	0
Pongola	16	29	0
Bwamba	13	37	0
Rift Valley Fever	1	4	0
Simbu	0	0	0
AR 344	0	0	0

The age distribution suggests that there was an epidemic spread of Chikungunya virus about 15 years before.

Sera collected from areas north and south of the Zambesi River were compared. Antibody to group A viruses tended to be commoner north of the river and Wesselsbron antibody was commoner south of the river. In sera from south of the Zambesi, antibody to all the viruses to which antibody occurred frequently was commoner in communities close to large rivers than at altitudes above 500 m. Details are given of the results for each individual locality.
C. E. Gordon Smith

WORTH, C. B., PATERSON, H. E. & DE MEILLON, B. **The Incidence of Arthropod-Borne Viruses in a Population of Culicine Mosquitoes in Tongaland, Union of South Africa (January, 1956, through April, 1960).** *Amer. J. Trop. Med. & Hyg.* 1961, July, v. 10, No. 4, 583–92, 1 fig. [14 refs.]

"From January, 1956, through April, 1960, 171,210 culicine mosquitoes from Ndumu, Natal, Union of South Africa, were inoculated into mice in 2,325 lots for attempted virus isolation. Fifty-nine recoveries of viruses were effected.

"The viral isolates, including nine identified viral types, were recovered from nine species of mosquito, but among these the only ones principally concerned were *Aedes* (*Neomelaniconion*) *circumluteolus* (Theobald), *Culex* (*Culex*) *univittatus* Theobald, *Mansonia* (*Mansonioides*) *africana* (Theobald) and *Mansonia* (*Mansonioides*) *uniformis* (Theobald), in the order named.

"The incidence of viral isolates in time and space at Ndumu indicates the possibility of the following assumptions having some basis in fact: (a) host specificity of most of the viruses concerned is not strict among genera of the tribe Culicini; (b) the virus types occurring in the Ndumu region may have undetected local cryptic cycles, their episodic reappearances being accounted for less by foreign reintroduction than by the occasional conjunction of ecological conditions that permits them to enter mosquito cycles detectable by current methods of investigation."

OSTERRIETH, P. M. & DELEPLANQUE-LIÉGEOIS, Paula. Présence d'anticorps vis-à-vis des virus transmis par arthropodes chez le chimpanzé (*Pan. troglodites*). Comparaison de leur état immunitaire à celui de l'homme. [**Antibodies to Arborviruses in Chimpanzees and Comparison of the Immunological State with that in Man**] *Ann. Soc. Belge de Méd. Trop.* 1961, Feb. 28, v. 41, No. 1, 63–72. [18 refs.]

Sera were collected from 65 chimpanzees from forest savannah in Ango and Bondo and from equatorial forest near Stanleyville in the Congo, also from 28 bought from Congalese. 84% of the animals were less than 5 years old. Neutralization tests were made by the method of SMITH and WESTGARTH [this *Bulletin*, 1959, v. 34, 1304]. Sera from persons aged under 20 years in the same areas were used for comparison. From the forest savannah 15% of chimpanzees and 16% of young people had Chikungunya antibody, 38% and 13% Semliki Forest antibody, 40% and 3% dengue-1 antibody, 56% and 19% dengue-2 antibody, 38% and 6% West Nile antibody, 62% and 12% yellow fever antibody, and 31% and 18% Bunyamwera antibody. From equatorial forest 7% and nil had Chikungunya antibody, neither had Semliki antibody, 16% and nil had dengue-1 antibody, 30% and 5% dengue-2 antibody, neither had West Nile antibody, 30% and nil had yellow fever antibody, and nil and 25% had Bunyamwera antibody. Arthropod-borne virus infections appear to be commoner in animals from savannah than forest. Even higher percentages of positives against dengue-2 (82%) and West Nile (53%) were found in bought chimpanzees, presumably resulting from additional exposure in the villages.

In group B 8·5% of sera were positive only against dengue-2, 5% only against yellow fever and 1% only against each of dengue-1 and West Nile. Group A infections (Chikungunya and Semliki) were much commoner in savannah. The presence of Semliki antibody in chimpanzees in this region was significantly associated with the presence of yellow fever and Bunyamwera antibody —presumably an ecological association. Group B infections were much commoner in chimpanzees than in man from the same areas. In forest Bunyamwera antibody was commoner in man than in chimpanzees, and *vice versa* in savannah. *C. E. Gordon Smith*

REEVES, W. C., BELLAMY, R. E. & SCRIVANI, R. P. **Differentiation of Encephalitis Virus Infection Rates from Transmission Rates in Mosquito Vector Populations.** *Amer. J. Hyg.* 1961, May, v. 73, No. 3, 303–15, 1 fig.

Studies were made of an endemic area of Western (WEE) and St. Louis (SLE) encephalitis in California from 1954 to 1959. *Culex tarsalis* were collected either in chicken-baited traps or from daytime resting places. Pools of not more than 50 mosquitoes were tested for virus either in mice or tissue cultures (chick embryo or hamster kidney). The chickens in the traps were tested for viraemia and later for neutralizing or haemagglutinin-inhibiting antibody.

In the 1954 season 37% of pools of engorged *C. tarsalis* yielded WEE and 20% SLE virus compared with 41% and 22% of pools of gravid *C. tarsalis* and 8% and 9% of pools of empty *C. tarsalis* respectively collected i the same shelters. The empties comprise mosquitoe which have not yet blood-fed and those which have fe and laid eggs but not yet fed since. From 1955 to 195 3–11% of gravid and engorged *C. tarsalis* pools yielde WEE and 0–4% SLE while of actively feedin *C. tarsalis* 1–5% and 0–2% yielded the respective viruses The mean number of *C. tarsalis* attracted to the chicken varied from 107 to 328 per bird in a single night an 30–95% of these fed. A much higher proportion fe when the chicken was restrained in nylon stocking tha when free in a cage. The WEE infection rates in th mosquitoes in the traps was always about 4 times th transmission rate and similar differences were foun with SLE virus. Many of the infected mosquitoes ha not yet reached the end of the extrinsic incubatio period.

The infection rate in chickens in traps was compare with the infection rates in nearby farm-flocks of chickens In 1958 11% of bait chickens became infected in a single night exposure but only 61·5% of flocks in the sam area were infected with WEE in the whole 4-mont season. In 1959 4% of bait chickens were infected wit SLE in a single night compared with 33% of nearb flocks. A single restrained bird appears likely to b attacked more frequently than free birds in flocks an infection rates tended to be higher in small flocks tha larger ones. *C. E. Gordon Smith*

BURGDORFER, W., NEWHOUSE, V. F. & THOMAS, L. A **Isolation of California Encephalitis Virus fron the Blood of a Snowshoe Hare (*Lepus amer canus*) in Western Montana.** *Amer. J. Hyg.* 1961 May, v. 73, No. 3, 344–9.

The blood of an emaciated sluggish snowshoe har (*Lepus americanus*) trapped in August 1959 wa inoculated into suckling mice half of which died withi 5 days. Passage of spleen and liver from these mic killed sucklings in 3 days. The agent was filterable (Seit EK) and highly sensitive to ether.

Large doses intracerebrally (IC) or intraperitoneall (IP) in suckling mice and deer mice (*Peromyscu maniculatus*) consistently killed in 2–3 days. IC inoculated adult mice died in 3–6 days but IP irregularl in 7–10 days. In adult mice maximum viraemia of 10 LD50 was detected 20–31 hours after infection. Sucklin and half-grown hamsters were susceptible to IC and I inoculation but intramuscular inoculation of the latte usually caused non-fatal paralysis. Viraemia as high a $10^{4·5}$ LD50 per ml. was found from 5–66 hours afte infection. Domestic rabbits showed no symptoms follow ing inoculation but had viraemia. A cottontail rabb (*Sylvilagus sp.*) inoculated also had viraemia. Chic embryos 6 days old died 2–5 days after inoculation an yolk sacs contained as much as $10^{7·1}$ mouse LD50 pe ml. Infectious mouse brain killed 50% of eggs at dilution of 10^{-7}.

No cytopathic changes were seen or virus multiplica tion demonstrated in inoculated KB, HeLa or Maben ce

cultures. Guineapigs, monkeys and chickens appeared to be insusceptible.

The hare from which the virus was isolated was heavily parasitized by *Dermacentor andersoni*: 131 of these nymphs and adults were kept alive for 2 months then ground up and inoculated but no virus demonstrated. Larva *D. andersoni* were fed on experimentally infected hamsters and examined for virus unsuccessfully at intervals from 1–10 days after feeding. Similar negative results were obtained with *Ornithodoros parkeri* and *O. turicata*. About 50 *Culex tarsalis* were fed on viraemic suckling mice: virus was recovered from them during the following 22 days but attempts to transmit by bite to mice were unsuccessful. In a similar experiment with *Aedes aegypti* virus was recovered and transmission by bite occurred once. The titre of virus in *A. aegypti* fell from an initial 2·1 log LD50 to less than 1 log LD50 after 4 days then rose to a maximum of 3·5 log LD50 on the 8th day, at which it remained for the following 17 days. Cross complement-fixation and neutralization tests with a wide range of arthropod-borne viruses showed that the new agent was identical with or closely related to California encephalitis virus. No haemagglutinin has been prepared. *C. E. Gordon Smith*

ZÓŁTOWSKI, Z. & WRÓBLEWSKA-MULARCZYKOWA, Zofia. Wstepne badania nad rolą komarów w przenoszeniu wirusa kleszczowego zapalenia mózgu. **[Preliminary Investigations on the Role of Mosquitoes in the Transmission of the Tick-Borne Encephalitis Virus]** *Med. Dośw. i Mikrob.* Warsaw. 1961, v. 13, No. 3, 241–9, 1 fig. [15 refs.]

The English summary appended to the paper is as follows:—

"In 1955–56 tick-borne encephalitis strains were isolated from mosquitoes in the Białowieża National Park. It was decided therefore to investigate the abilities of tick-borne encephalitis virus to survive in certain mosquito-species (Aedes (O.) communis (Deg.), Aedes (O.) excrucians (Walk.) Anopheles messeae (Fall.).

"The mosquitoes were infected using the method elaborated by Ross (with some modifications). They were stored at 4°C, every 12—24hrs.; pools of 8—10 insects were ground and the suspensions titrated intracerebrally in mice.

"It was established, that in mosquitoes Aedes (O.) communis (Deg.) and Aedes (O.) excrucians (Walk) species the virus could be found up to 60hrs. after infection, whereas in the species Anopheles messeae (Fall.) only for 36hrs.

"An attempt was made to investigate the possibilities of mechanical transfer of the virus applying the so called "interrupted sucking". The insects were partially fed a virus suspension in the Ross-apparatus, their sucking interrupted and set up again on a healthy mouse. For a successful transmission, the following optimal conditions seem to be necessary: a high titer virus suspension, short intervals between sucking and at least 8 mosquitoes for infecting 1 healthy mouse."

SALMINEN, A., ERIKSSON, A. W. & OKER-BLOM, N. **Hemagglutination-Inhibiting Antibodies in the Human Population of an Endemic Area of Diphasic Tick-Borne Meningoencephalitis.** *Arch. f.d. gesamte Virusforschung.* 1961, v. 11, No. 2, 215–23, 4 figs. [14 refs.]

"Sera from 716 inhabitants of the Åland archipelago were examined for hemagglutination-inhibiting antibodies with the diphasic tick-borne encephalitis virus. The antibodies were found in 13.1 per cent of the whole series. There was no significant difference in antibody rate between males and females. It was noted that the antibody rate of the population increased almost linearly with age, which indicates that the human population of the area is exposed to a relatively constant risk of infection. Great local variations in the incidence of the antibodies were found among the residents of neighboring islands. No accumulation by families could be established in the distribution of immunity, which speaks against transmission of the virus through milk in the area."

RAPELLINI, M. & SARDI, P. I cortisonici nel morbillo. **[Cortisones in Measles]** *Giorn. di Malattie Infettive e Parassit.* 1961, Mar., v. 13, No. 3, 164–6.

The authors report on 800 patients with measles in the Infectious Diseases Hospital, Turin, admitted during 1958–60, who developed complications involving either the bronchopulmonary or the cerebrospinal systems.

Bronchopulmonary complications were seen in 78 patients or 10%, mostly children aged up to 5 years, and the manifestations usually appeared within 3 days of the rash; 2 died. The patients were given cortisone drugs, usually the acetate by injection, 4 times a day, continued for 4 days. The daily dosage was 3–5 mgm. per kgm. of body weight. Tetracycline or chloramphenicol was given at the same time, and the temperature subsided generally by crisis within 48 hours, with relief of the dyspnoea and of the toxic condition.

The cerebrospinal complications took the form of polyneuritis in 1 patient, acute psychosis in 2, meningoencephalitis in 13 and encephalitis in 8—24 patients in all or 3% of the series. These patients also received antibiotic treatment together with a daily dosage of 5 mgm. of cortisone per kgm. of body weight for the somewhat longer period of 6 days. Pyrexia subsided in 60–72 hours, the other, cerebrospinal, manifestations abating a few days later. A starting intravenous dose of 2·5 mgm. per kgm. of hydrocortisone hemisuccinate, repeated after 12 hours, was given to 2 patients with very high fever (temperature 41·8°C.). One patient died with hyperpyrexia. A girl of 22, from the mountains, developed the rash of measles shortly after having been admitted to a mental hospital: she recovered from the measles but not from her mental derangement, which is hardly likely to have been due to the exanthem.

J. Cauchi

OKUNO, Y., SUGAI, T., TOYOSHIMA, K., TAKAHASHI, M., YAMAMURA, T., HATA, S. MIKI, T., NAKAMURA, K., UEDA, S. & KUNITA, N. **Studies on the Prophylaxis of Measles with Attenuated Living Virus. IV. Inoculation Tests in Children with Chick Embryo Passage Measles Virus in 1960.** *Biken's J.* Osaka. 1960, Dec., v. 3., No. 4, 293–300. [16 refs.]

Vaccination studies have been carried out in children with 2 lines of the Toyoshima strain of measles virus passaged in the amniotic and allantoic cavities respectively. 937 children living in an isolated village which had been unaffected by measles for several years were given 2nd-3rd chorioallantoic passage virus by aerosol inhalation; none developed any symptoms. Formation of measles complement-fixing antibody in titres up to 256 was observed in 13 of 67 children. 22 children aged between 7 months and 4¾ years who were living in a group of apartment houses were similarly vaccinated with virus which had been through 26 amniotic and 6 allantoic passages. 19 developed fever of 1–6 days' duration after an incubation period of 6–11 days; 8 of these children developed a rash, which was less marked and less extensive than the rash of natural measles. Catarrh, cough, conjunctival hyperaemia and Koplik spots were not observed. The appearance of measles complement-fixing and neutralizing antibody was observed in 7 children who were studied serologically.

D. J. Bauer

FOWLER, R. S. & BAILEY, J. D. **Cat Scratch Disease in Childhood.** *Canadian Med. Ass. J.* 1961, June 17, v. 84, No. 24, 1365–8. [11 refs.]

In the study here described the findings in 55 cases of cat scratch disease in children diagnosed during 1955–60 in the Hospital for Sick Children, Toronto, are summarized.

There were 24 girls and 31 boys in this patient group ranging in age from 2 to 14 years and 39 were between 5 and 9 years. The highest incidence was during the winter months. In 22 cases there was a definite history of being scratched by a cat and 13 patients had primary lesions in the appropriate area from other causes which are listed.

Fever occurred in 41 of the children but as a rule was not high and only 18 had general complaints of illness. A list is given of the sites of the glandular enlargement which was most frequent in the axilla (23 cases). Suppuration of the involved glands occurred in 23 cases and was usually treated by needle aspiration or surgical incision and drainage.

Other observations in some of these children were palpable spleen (5 cases), erythema nodosum (2), arthralgia (1) and pityriasis rosea (1).

The findings of blood tests and bacteriological examinations are recorded and followed by a discussion The features which suggest a diagnosis of cat scratch disease are (1) regional lymphadenopathy, (2) history of contact with a cat, (3) a dull red, indurated and crusted papule in the area drained by the gland, (4) no signs or laboratory evidence of other diseases causing lymphadenopathy, (5) a low white blood cell count, (6 eosinophilia of 5% or more and (7) a positive skin tes with a potent cat scratch antigen. *M. E. Delafield*

ERGAS, M. **[Acute Infectious Lymphocytosis. Repor of 11 Cases]** *Harefuah.* Jerusalem. 1961, July 2 v. 61, No. 1 [in Hebrew 10–13, 2 figs. (20 refs English summary 13–14.]

The English summary appended to the paper is a follows:—

"Eleven cases of acute infectious lymphocytosis ar described from 4 hospitals in Israel over the perio 1953–60.

"The children were from 1–11 years of age. In 1 cas there were no clinical signs; 4 cases had diarrhea vomiting and abdominal pain; 2 cases had signs of uppe respiratory infection; 2 cases had convulsions an unconsciousness and 2 cases had limb pains.

"Blood counts showed 26,000—100,000 leukocyte with 50—90% lymphocytes of normal appearance. In case the lymphocytes were smaller than normal and i 1 they were larger. Bone marrow examinations showe an increase of lymphocytes.

"The etiology, epidemiology and differential diagnosi of the disease are discussed. In all cases the course o the disease was benign and without complications."

MUŞETEANU, C., MUŞETEANU, Valentina, PARASCHIVESCU N. & VIŞINESCU, D. Asupra unei boli eruptiv datorite unui virus mare. **[An Exanthematic Ill ness Caused by a Large Virus]** *Studii si Cercetăr Inframicrobiol.* 1961, v. 12, No. 1, 105–7. Frenc summary (3 lines).

In 1957 the authors observed an outbreak of previously unknown illness accompanied by an exanthem The first case occurred in spring. The onset was acut with fever, headache, and a generalized eruption consis ing of pink macules 2–3 cm. in diameter of irregula outline. The illness lasted 7 days and the exanthem disappeared without desquamation. Convalescence wa protracted. The same syndrome was also observed in boy aged 9, and in the second week of his illness hi brother aged 3 years also contracted the disease.

Similar cases were observed between June and Octobe and in 14 instances an infective agent resembling rickettsia could be isolated. In preparations from th yolk sac of fertile eggs inoculated on the 7th day o incubation with blood from patients, coccoid or cocco bacillary organisms 200–300 mμ in size could b observed, which stained pink with Giemsa. Th organisms could be carried through 15 passages in egg and when inoculated into mice intraperitoneally ascite developed with mononuclear cells containing larg numbers of the organisms.

In 2 patients aged 4 and 12 years the disease was fata and the same agent could be recovered from the organ at autopsy. *D. J. Bauer*

Bacteriology and Immunity

The order in this section is:—**General; Bacteriostatic and Bactericidal Substances; Bacteria; Rickettsiae; Bacteriophages; Viruses; Immunity.**

Abstracts of papers relating to the bacteriology of plague, cholera, yaws, leprosy and certain other tropical bacterial diseases; of the rickettsial fevers; and of yellow fever, dengue, rabies and certain other tropical virus infections, are published in the TROPICAL DISEASES BULLETIN. *Their titles and page references are cited below in the appropriate sub-divisions of this section.*

GENERAL

STEEL, K. J. & FISHER, P. J. **A Fallacy of the Nitrate Reduction Test.** *Monthly Bull. Ministry of Health & Pub. Health Lab. Service (directed by Med. Res. Council).* 1961, Mar., v. 20, 63–5.

The usual test for the reduction of nitrate by bacteria depends on the development of a red colour when the naphthylamine-sulphanilic-acid reagent is added to a broth culture of the organism under test. Before inoculation, the broth culture medium must contain nitrate but no nitrite. The absence of the red colour change does not necessarily mean failure to reduce nitrate. In order to determine whether or not the original nitrate in the uninoculated broth has, after inoculation and incubation, been utilized by the growing organism, zinc dust may be added in order that any residual nitrate may be reduced to nitrite so that the test reagents will give the red colour change. If at this stage there is still no development of the colour change in the presence of the naphthylamine-sulphanilic-acid reagent it may be assumed that the original nitrate had been reduced to nitrite but that further reduction of the nitrite had occurred. It is important that not more than 5 mgm. of zinc dust per ml. of culture fluid is added otherwise the red colour may quickly fade or it may even be inhibited. *H. J. Bensted*

NICHOLAS, D. J. D., SILVESTER, D. J. & FOWLER, J. F. **Use of Radioactive Nitrogen in studying Nitrogen Fixation in Bacterial Cells and their Extracts.** *Nature.* 1961, Feb. 25, v. 189, 634–6. [12 refs.]

Radioactive nitrogen (Nitrogen-13, $\beta + 1{\cdot}24$ MeV.; half-life 10·05 minutes) was used to study nitrogen fixation by (i) whole cells of *Azotobacter vinelandii* (O strain) and (ii) cell-free extracts. For optimum fixation the gas had to contain 10% oxygen and exposure of the cell systems to the gas for 10 minutes gave substantial nitrogen uptake. In the cell-free extracts, cell particles were necessary to sustain the uptake of nitrogen-13. Labelled compounds could be detected and these are being further studied. By using N-13 instead of N-15 in studying fixation, less time is required for the experiments. The paper must be consulted for technical details. *Elizabeth M. Harper*

MARMUR, J. & SCHILDKRAUT, C. L. **Growth of Bacteria labelled with Heavy Isotopes for the Isolation of Nucleic Acids.** *Nature.* 1961, Feb. 25, v. 189, 636–8, 1 fig.

This paper describes a method of labelling homogeneously the deoxyribonucleic acid (DNA) of bacteria with the heavy isotopes of hydrogen (D) and nitrogen (^{15}N). DNA so labelled has a higher density than unlabelled DNA and can be distinguished from it in studies of the interactions of nucleic acids and their genetic fate and function by density measurements by means of the caesium chloride density gradient centrifugation technique (MESELSON, *et al., Proc. U.S. Nat. Acad. Sci.* 1957 v. 43, 581).

Labelling is achieved by growing bacteria with simple nutritional requirements in a medium consisting of a variety of mineral salts+glycerol+sodium lactate. $^{15}NH_4Cl$ is the sole source of nitrogen and all the constituents are dissolved in heavy water (D_2O) of more than 99% purity. A less-complete labelling of organisms with more exacting nutritional requirements can be obtained by replacing the water in their normal medium with heavy water. In the use of this technique two problems have to be overcome. Firstly in initial growth bacteria take up hydrogen from ordinary water in preference to deuterium from heavy water. This is dealt with by dissolving the main ingredients of the growth medium in 10 ml. of heavy water, evaporating down to 1 ml., and finally making up to 100 ml. with heavy water + trace elements. Sterilization is by filtration to avoid the introduction of ordinary water. The second problem is the lack of growth of bacteria exposed suddenly to high concentrations of heavy water. Adaptation is secured by starting growth in a small quantity of medium containing one-third ordinary water and two-thirds heavy water and making successive additions of heavy water medium during growth.

This D_2O-^{15}N medium supported growth and gave homogeneously labelled DNA for the bacterial species *Alcaligenes faecalis, Salmonella typhi-murium, Pseudomonas pyocyanea, Klebsiella pneumoniae, Bacillus cereus, Bacillus subtilis, Escherichia coli,* and *Serratia marcescens* [*Chromobacterium prodigiosum*]. *Streptococcus pneumoniae* was grown in a complex medium containing 99% heavy water. The DNA was isolated from cells of each of these species and the densities in caesium chloride were determined. A table gives a comparison of the densities of normal and labelled DNA. The densities of *E. coli* DNA labelled with ^{15}N, D and ^{15}N-D are also given. A prediction was made of the densities of variously labelled DNA based on the percentage increase in molecular weight of nucleotides brought about by substituting ^{15}N and D for N and H respectively. With the 3 species from which ^{15}N-labelled DNA was isolated the predicted density shift

was found to occur. So far it has not been possible to secure 100% labelling with deuterium since the medium contains H in glycerol and sodium lactate but it is intended to use completely labelled acetate as sole carbon source to see whether the actual and predicted density shifts for D-labelled DNA agree.

The authors mention that they have used ^{15}N-D-labelled DNA extensively in physical-chemical studies of strand separation and recombination and in the formation of " hybrid " DNA molecules. They suggest that the procedure described might be used for such purposes as the preparation of " heavy " whole cells, phage, proteins, RNA and other cell constituents.

R. B. Morrison

SCHAEFFER, P., EDGAR, R. S. & ROLFE, R. Sur l'inhibition de la transformation bactérienne par des désoxyribonucléates de compositions variées. [**Inhibition of Transformation of Bacteria by DNA of Various Composition**] *C.R. Soc. Biol.* 1960, v. 154, No. 11, 1978–83. [17 refs.]

The transformation of bacteria by DNA from another strain is inhibited competitively by the presence of DNA from the strain being transformed (the receptor strain) [ALEXANDER *et al., J. Exper. Med.*, 1954, v. 99, 505; this *Bulletin*, 1954, v. 29, (996)]. To see if DNA with the same base composition as the receptor strain, or DNA derived from closely related species, inhibits transformation, DNA from various bacteria was mixed with transforming DNA from a streptomycin-resistant mutant of *Haemophilus influenzae* strain Rd, the mixture was incubated with *H. influenzae* Rd cells and the degree of inhibition of transformation was measured. No correlation was found between the inhibiting activity of a DNA and either the ratio of its bases or the taxonomic relationship of donor and receptor strains. For example, DNA with the same base ratio as that of *H. influenzae* did not inhibit transformation when derived from *Actinobacillus equuli* or *Bacillus megatherium* but did inhibit when derived from *H. parainfluenzae* A61. On the other hand, DNA with different base ratios but derived from such unrelated species as *Staphylococcus aureus* and *Micrococcus lysodeikticus* inhibited transformation.

The authors explain their findings by suggesting that DNA from different sources is active in transformation or its inhibition because it possesses the same structure in a relatively small part of its molecule, such as, for instance, in the sequence of terminal nucleotides.

Janice Taverne

KUIDA, H., GILBERT, R. P., HINSHAW, L. B., BRUNSON, J. G. & VISSCHER, M. B. **Species Differences in Effect of Gram-Negative Endotoxin on Circulation.** *Amer. J. Physiol.* 1961, June, v. 200, No. 6, 1197–202, 5 figs. [17 refs.]

CONNOR, D. G. & KASS, E. H. **Effect of Artificial Fever in Increasing Susceptibility to Bacterial Endotoxin.** [Correspondence.] *Nature.* 1961, April 29, v. 190, 453–4.

When mice are maintained at a temperature of 22°C throughout the experiment it is found that the LD50 of an endotoxin prepared from typhoid bacilli injected intraperitoneally is 400–800μgm. If mice kept at 22°C are given endotoxin and then kept at 37°C. the LD50 is 21μgm., but if the mice are preincubated at 37°C given the endotoxin and then kept at 37°C. the LD50 is less than 1μgm. Thus mice maintained throughout at 37°C. will respond to 1 gm. of endotoxin by a rise in body temperature to more than 41·5°C. with death of two-thirds of the animals within 3 hours of receiving the endotoxin. It is therefore very important that there should be careful control of the body temperature of all animals used in any experimental work involving the administration of lethal doses of endotoxin.

H. J. Bensted

SMITH, H. W. & CRABB, W. E. **The Faecal Bacterial Flora of Animals and Man: its Development in the Young.** *J. Path. & Bact.* 1961, July, v. 82, No. 1, 53–66, 5 figs.

BACTERIOSTATIC AND BACTERICIDAL SUBSTANCES; DISINFECTION

The order in this section is:—**General; Antibiotics; Colicines; other Therapeutic Substances; Disinfection.**

WORLD HEALTH ORGANIZATION. TECHNICAL REPORT SER. No. 210. **Standardization of Methods for conducting Microbic Sensitivity Tests. Second Report of the Expert Committee on Antibiotics. Geneva, 11–16 July, 1960.** 24 pp. 1961. Geneva: Palais des Nations. [Sales agent for U.K., H.M. Stationery Office.] [1s. 9d.; $0.30; Sw.fr. 1.–.]

The adoption of universally accepted standardized methods for testing the sensitivity of bacteria to antibiotics would afford the best guidance to clinicians in the treatment of patients and allow comparisons of the frequency and the degree of resistence in bacterial species in different institutions and countries. The description of a micro-organism as " sensitive " or " resistant " to an antibiotic is inexact; and it is preferable to state the concentration to which the organism is sensitive. Since there is at present no standard method the technique should be described.

In a section defining the nature of resistance, the

importance of heterogeneity is stressed. Ideally, the sensitivity analysis of a culture should show the frequency distribution of individual strains as a function of the maximal tolerated concentration. In any sufficiently large microbial population resistant clones will emerge as the result of selection especially during the treatment of a chronic infection with an antibiotic.

It is suggested that for routine tests for the common pathogenic bacteria (excluding tubercle bacilli) the following 10 antibiotics should be used, erythromycin, novobiocin, vancomycin, bacitracin, penicillin, tetracycline, chloramphenicol, neomycin, streptomycin and polymyxin. The penicillin used should be G, but when a different form is being used clinically this should also be tested; in the tetracyclines cross-resistance is complete and it is sufficient to test the organism against tetracycline. Drugs of the neomycin group, namely, neomycin, kanamycin, framycetin and paromomycin are related and for most purposes only one need be tested.

The recommendation is made that the standard method employed in routine clinical practice should be a diffusion method in which filter-paper discs containing the antibiotic are applied to the surface of an inoculated culture medium in a plate. This method is economical in labour and can be made to yield a sufficiently accurate quantitative result for clinical purposes. Except in large laboratories with highly skilled staff, commercially prepared paper discs should be used. It is desirable that there should be government control over the preparation of the discs especially with regard to their antibiotic content. There follow recommendations with regard to conditions which should be observed in the performance of the test with regard to the quantity of antibiotics in the disc, the medium containers, inoculum, and reading of results.

Tests for combined antibiotic action are not defined but the view is expressed that to assess bactericidal action for clinical purposes the most helpful method may be that in which tubes of liquid cultures containing mixtures of antibiotics are subcultured on a solid medium to estimate the proportion of survivors.

No details of methods for *Mycobacterium tuberculosis* are given in this report.

[This is an attempt to convey some of the important points raised but the contents cannot satisfactorily be summarized and the report should be read in full by all concerned with the performance of antibiotic microbial sensitivity tests.] *D. D. Smith*

KELSEY, J. C. **The Testing of Sterilizers. 2. Thermophilic Spore Papers.** *J. Clin. Path.* 1961, May, v. 14, No. 3, 313–19, 5 figs.

A method is described for the preparation of spore suspensions of thermophilic bacteria. By a process termed bulk loading the spores are transferred to Whatman's antibiotic-assay discs which are of special paper, 6 mm. in diameter and absorb 0·033 ml. of water. A statistical experiment showed that this method gives an even distribution of spores on the dried discs. The statistical method was adopted when the quantitative recovery of individual spores by disintegrative techniques had failed. The belief that spore papers must be subcultured into a specific medium immediately after use is shown to be false.

The methods used for estimation of the heat resistance of bacterial spores depend upon the estimation of the number of spores surviving a given heat treatment. In this study, quantitative spore recovery being impossible, it was decided to treat each spore paper as a biological unit, and in this way survivor/time graphs were constructed. These were sigmoid, the gradient of the curve being a characteristic of the bacterial strain and indicating that some strains are more suitable than others for the testing of sterilizers. The LD50 to moist heat was thus determined for each strain.

This simple method gives the same value for the LD50 as does a more refined statistical analysis.

On the basis of this work the author suggests 5 minutes as being a suitable value for the LD50. The nature of the curve is such that the probability of a single paper being sterile after less than 5 minutes at 121°C. can be overcome by using more than one. As the LD50 of the spore papers is governed by the number of spores on each paper, it can be adjusted at will.

The author concludes that carefully standardized spore papers should be used only by experienced workers, Browne's tubes being preferable for routine use. *E. Stuart Broughton*

BACTERIA

The order in this section is:—**Cocci; Donovania; Mycobacterium; Corynebacterium; Haemophilus; Pseudomonas; Pfeifferella; Enteric Organisms; Brucella; Pasteurella; Bacillus; Anaerobes; Spirochaetes; Erysipelothrix; Listeria; Others.**

BATTISTINI, F. Ricerche sulla aloresistenza e aloassuefazione delle tre varietà di stafilococchi. [**Resistance and adaptation to Salt of the Three Varieties of Staphylococci**] *Giorn. di Batteriol., Virol. ed Immunol.* 1961, Jan.–Apr., v. 54, Nos. 1/4, 13–20. [10 refs.] English summary.

37 strains of *Staph. aureus*, 4 of *Staph. citreus*, and 79 of *Staph. albus* cultivated from 250 samples of ice-cream, not associated with food poisoning, were studied to see (1) their power to grow in a liquid medium containing 7·5% NaCl, (2) their adaptation and resistance to salt by passage in a liquid medium containing increasing NaCl concentrations up to 13%, (3) their power of fermenting lactose and mannite in these salt concentrations, and (4) the coagulase reaction of strains so cultivated.

All the strains grew in Chapman's medium with

7·5% NaCl. Successive subcultures onto broth containing 9, 11, and 13% NaCl showed that half the *albus* strains, almost all the *aureus* and 1 out of 4 *citreus* resisted up to the highest concentration. Almost the same result was obtained when the strains were seeded directly into 13% NaCl broth. Out of 28 *aureus* strains, 26 retained their power to ferment lactose and 23 that of fermenting mannite after adaptation to growth in 13% NaCl broth; out of 33 *albus* strains 26 and 28 retained their lactose and mannite fermenting properties respectively. When the strains were subcultured on to 13% NaCl broth directly from 7·5% broth, 26 out of 65 strains lost their fermentative properties. 13 out of 79 *albus* strains, 14 out of 37 *aureus* and 1 out of 4 *citreus* were coagulase positive, these being among those which showed resistance and adaptation to salt. *E. Agius*

LEWIS, B. **Phosphatase Production by Staphylococci —A Comparison of Two Methods.** *J. Med. Lab. Technol.* 1961, Apr., v. 18, No. 2, 112–13.

Unselected strains of staphylococci were tested for phosphatase production by the plate method [BARBER and KUPER, this *Bulletin*, 1951, v. 26, 944] and in broth containing phenolphthalein diphosphate at about 1 in 10,000. Broths were tested for pink coloration on addition of 1 drop of 40% sodium hydroxide after 6 hours' and 18 hours' incubation. Plates were tested for pink coloration by exposure to ammonia vapour after 18 hours incubation.

By the plate method 42 of 44 coagulase-positive strains were phosphatase (P) positive but 31 of 56 coagulase-negative strains were also P–positive. By the broth technique 41 of 44 coagulase-positive strains were P–positive after 6 hours while only 1 of 56 coagulase-negative strains was positive. After 18 hours' incubation, however, 31 other coagulase-negative strains became P–positive.

[There is no indication that strains which were coagulase-negative and P–positive by the plate method could be distinguished by any other means such as intensity of coloration or colonial appearance. The advantage of using the phosphatase reaction in the plate method for sampling large amounts of material containing a mixed flora is lost if organisms have to be isolated in pure culture as in the tube test.]

M. Patricia Jevons

MOUSTARDIER, G., DULONG DE ROSNAY, C., DU PASQUIER, P. & LATRILLE, J. Influence de la protamine sur la morphologie des bactéries. [**Influence of Protamine on the Morphology of Bacteria**] *Ann. Inst. Pasteur.* 1961, Aug., v. 101, No. 2, 192–202, 9 figs. on pl. [15 refs.]

The English summary appended to the paper is as follows:—

"Protamine sulfate, apart from its antibacterial properties and its protective action on protoplasts, may induce abnormal bacterial forms.

"The authors study these forms in staphylococci and call them protoplasts. They resemble the forms induced by lysozyme and penicillin, but differ from them by:

"1. Their resistance to osmotic shock.

"2. Their progressive and often incomplete reversion.

"3. Their longevity and the fact that their subcultures are indefinitely possible in protamine media."

HUTCHISON, J. G. P. **Crystals in Colonies of *Staphylococcus aureus*.** *J. Path. & Bact.* 1961, July, v. 82, No. 1, 214–17, 6 figs. on pl.

"Intracolonial crystals of calcium phosphate are commonly formed in ageing cultures of *Staphylococcus aureus* (coagulase-positive). Experiments suggest that a local rise in *p*H is responsible for the phenomenon and also for a curious circular distribution of crystals within the colonies."

HERTZ, Constance S. & WAITE, Barry F. **Observations on the Ammonium Molybdate Test for the Virulence of Staphylococci.** *Amer. J. Clin. Path.* 1961, May, v. 35, No. 5, 472–4. [Reprinted from *Tech. Bull. Registry Med. Technol.* 1961, Apr., v. 31, No. 4, 70–72.]

The ammonium molybdate test (AMC) [this *Bulletin*, 1959, v. 34, 939] was compared with coagulase production, pigment formation, mannitol fermentation and haemolysin production as a test for virulence of staphylococci. 100 strains from urine, sputum, nasal secretions, and various lesions were tested.

For the AMC test 0·08 ml. of 2·5% ammonium molybdate solution was added to 24–hour cultures of the organisms in 0·5 ml. rehydrated plasma. After shaking, 0·04 ml. Pictol [P-methylaminophenol sulphate] reagent and 0·1 ml. 2% HCl were added. Positive cultures developed a blue colour in 15 minutes.

The correlation between coagulase production and a positive AMC test was better than that between coagulase production and any other of the tests. All AMC-positive strains were coagulase-positive and all coagulase-negative strains were AMC-negative. 3 strains were AMC-negative and coagulase-positive.

White mice were infected intraperitoneally with 0·25 ml. broth culture of various strains. Mice were killed after 7 days and the staphylococci isolated from blood, liver, spleen and kidney were tested by the AMC test and for coagulase production. Of 34 coagulase-positive and AMC-positive strains 26 (76%) were recovered and only 12 remained coagulase-positive and AMC-positive. Both coagulase-positive and AMC-negative strains were coagulase-negative on recovery. Results of tests were unchanged on the 11 strains which were recovered out of 14 coagulase-negative and AMC-positive strains tested. [No death rates were given and strains were not phage typed before and after mouse passage.]

20 preparations of coagulase, devoid of hyaluronidase when tested by the mucin clot prevention test, were AMC-positive and the addition of hyaluronidase to coagulase-negative strains did not alter the AMC reaction in previously AMC-negative strains.

The authors conclude that the AMC test has no advantage over the coagulase test as a test for pathogenicity of staphylococci.

M. Patricia Jevons

SWARTZ, M. N., BLACK, P. H. & MERSELIS, J. **Effect of *Staphylococci* on Leucocyte Pyridine Nucleotides.** *Nature.* 1961, May 6, v. 190, 516–18, 2 figs.

It was postulated that the survival of *Staphylococcus aureus* in leucocytes might be due to activation of the coccal diphosphopyridine nucleotide pyrophosphatase. Experiments showed, however, no good correlation between the presence of activated DPN pyrophosphatase in the cocci and decrease of DPN in the leucocytes, though such decrease commonly occurred after phagocytosis had taken place.

R. E. O. Williams

JAMES, Ruth C. & MACLEOD, C. M. **Induction of Staphylococcal Infections in Mice with Small Inocula introduced on Sutures.** *Brit. J. Exper. Path.* 1961, June, v. 42, No. 3, 266–77, 8 figs. on 2 pls.

Silk threads were infected with staphylococci by soaking in appropriate dilutions of a broth culture. They were allowed to dry and then introduced under the depilated skin of a mouse's back; the ends of the sutures were cut and capped with collodion. Abscesses developed in 3–4 days. The smallest dose of staphylococci that consistently produced abscesses was about 1,000 viable units, estimated by shaking the sutures in broth and performing a viable count on the broth suspension. The dose leading to pus formation in 50% of the animals was rather less than 10 organisms for a type 80/81 strain and between 10 and 100 for a group of other strains of coagulase-positive staphylococci. Definite pus formation was not seen with coagulase-negative strains.

In a proportion of the mice the sutures were lost from the skin within the first 1–2 days; loss of the suture greatly reduced the frequency of abscess formation, especially with small doses of staphylococci.

A second challenge to mice that had recovered from abscesses gave similar results, with no evidence of acquired immunity.

Various suture materials other than silk were also used, including cotton, dacron, nylon, steel, silicone-treated silk and human hair. There were differences in the extent to which different materials picked up the cocci, but with a dose of 10^3 cocci, abscess formation occurred in almost all animals with several of the suture materials. The "potentiating" effect of various materials seemed to be related to their ability to induce an inflammatory response when introduced without cocci. Determination of the 50% end-point was made difficult by the errors involved in counting small numbers of organisms and by the fact that sutures carrying small inocula were liable to be lost more often than those carrying large inocula.

R. E. O. Williams

COUNTS, G. W., SMITH, I. M., ROUTH, J. I., HAZARD, E. C. & MCTAVISH, J. F. **Biochemical Changes in Lethal Staphylococcal Infections in Mice.** *Nature.* 1961, Aug. 19, v. 191, 783–6, 2 figs.

VÁCZI, L. & FODOR, M. **The Relation of Surface Properties and Antibiotic-Resistance in *Staphylococcus aureus*. III. The Effect of Unsaturated Fatty Acids on the Resistance to Antibiotics.** *Acta Microbiologica.* Budapest. 1961, v. 8, No. 1, 43–51, 5 figs. [14 refs.]

The studies of the senior author and his colleagues on the bacterial surfaces of antibiotic sensitive and resistant strains of *Staph. aureus* are continued [this *Bulletin*, 1960, v. 35, 491, 914] by investigating the effect of unsaturated fatty acids on the sensitivity of these organisms to penicillin.

Oleic acid, linolenic acid and linoleic acid inhibited both the antibiotic sensitive and resistant strains tested, in concentrations over 0·12%, 0·03% and 0·03% respectively. Subinhibitory concentrations of the 3 fatty acids added to agar increased the penicillin sensitivity of penicillin resistant and polyresistant strains as judged by the disc technique but the sensitivity of a penicillin sensitive strain was unaffected. Subinhibitory concentrations of penicillin and sodium oleate together markedly reduced the oxygen consumption of the antibiotic resistant strains but only slightly reduced that of the sensitive strains. This synergic effect was not due to direct action on penicillinase. These findings are discussed in terms of the differences in lipid composition and metabolism of antibiotic resistant and sensitive staphylococci.

M. Patricia Jevons

GANOR, S. & TAS, J. **Sensitivity of Coagulase-Positive Staphylococci. Study in Various Forms of Pyoderma.** *Arch. Dermat.* 1961, June, v. 83, No. 6, 995–1001. [19 refs.]

The authors investigated the antibiotic patterns of coagulase-positive staphylococci isolated from patients who attended the skin department of the Hadassah University Hospital in Israel with pyodermia. The results were analysed with respect to the type of lesion, and previous contact with hospital, either of the patient himself, or other members of the family.

Of 177 strains from 239 patients, 22% were sensitive to penicillin, 94% to chloramphenicol and 88% of 135 strains were sensitive to erythromycin. The resistance rate of strains from patients who had been in hospital within the last 12 months was higher than that of strains from patients who had a member of the family in hospital and both these resistance rates were higher than that of strains from the rest of the outpatients. No difference was detected between strains isolated from impetigo or boils.

There was a higher resistance rate to penicillin and tetracyclines in strains from patients in whose family a baby had been born in hospital within the last 12 months and the authors suggest that the higher incidence of resistant strains in females than males that

they found, reflects the greater contact of women with these babies. There was also a significant increase in tetracycline-resistant strains in 1959 compared with strains isolated in a parallel period in 1958.

M. Patricia Jevons

SUGANUMA, A. **The Plasma Membrane of *Staphylococcus aureus*.** *J. Biophysic. & Biochem. Cytol.* Baltimore. 1961, June, v. 10, No. 2, 292–8, 10 figs. [18 refs.]

SNYDER, I. S. & HAMILTON, T. R. **Production of Streptolysin S by Streptococci Before and After Mouse Passage.** *Proc. Soc. Exper. Biol. & Med.* 1961, Apr., v. 106, No. 4, 836–9.

"(1) Streptolysin S was produced by streptococci of Lancefield groups A, C and G. (2) Streptococci of group A produced considerably more streptolysin S than did groups C and G. (3) Strains of group C streptococci of human origin produced more streptolysin S than did animal strains of group C. (4) In general, the passage of beta hemolytic streptococci through mice enhanced production of streptolysin S but did not enhance their virulence. Nonhemolytic strains were not affected by animal passage."

STOCK, A. H. & LYNN, R. J. **Preparation and Properties of Partially Purified Erythrogenic Toxin B of Group A Streptococci.** *J. Immunology.* 1961, May, v. 86, No. 5, 561–6, 1 fig. [19 refs.]

"Crude concentrates containing erythrogenic toxin B were prepared from filtrates or supernatants of cultures of three group A streptococci and the partially purified toxins were studied. Toxin B, like toxin A, appeared to be protein and was not precipitable by protamine. Toxin B, unlike toxin A, was largely destroyed in the presence of trypsin. Toxin B, in contrast to toxin A, did not flocculate with anti-A antitoxin. In agar gel diffusion tests, a specific precipitin reaction of toxin B-anti-B antitoxin was not identified."

NELSON, J. R. & WHITE, A. G. C. **Inhibition of Growth of Streptococci by Adenine.** *Proc. Soc. Exper. Biol. & Med.* 1961, June, v. 107, No. 2, 242–4.

"Adenine, at a concentration of 5 μg/ml or above in a synthetic medium, has been shown to inhibit growth of 2 strains of *S. pyogenes*, S23 and C203U. The inhibition can be overcome by addition of xanthine, guanine or hypoxanthine at approximately one-third the molar concentration of the inhibitor."

BERGER, U. Untersuchungen über die Pigmentbildung durch Neisserien. [**Studies on Pigment Formation by Neisseriae**] *Ztschr. f. Hyg. u. Infektionskr.* 1961, v. 147, No. 5, 461–9, 10 figs. [11 refs.]

TACQUET, A. & TISON, F. Nouvelle technique d'isolement des mycobactéries par le lauryl-sulfate de sodium. [**A New Technique for the Isolation of Mycobacteria by means of Sodium Lauryl Sulphate**] *Ann. Inst. Pasteur.* 1961, May, v. 100, No. 5, 676–80.

In a previous paper [this *Bulletin*, 1961, v. 36, 799] the authors reported the successful use of 5% Teepol 410 in 1% sodium hydroxide for treating specimens before cultivation for tubercle bacilli. Teepol has, however, the disadvantage that it is not a chemical entity and is therefore subject to variation. In the present paper the authors present evidence to show that this anionic detergent can be effectively replaced with 4% sodium lauryl sulphate.

They show:—(1) that quantities as high as 2 mgm of sodium lauryl sulphate on the surface of egg media do not interfere with growth of H37Rv; (2) that the mixture homogenizes sputum as well as the original Teepol mixture and better than either 5% Tween 80 in 1% sodium hydroxide or sodium hydroxide alone; (3) that the mixture effectively kills the non-mycobacteria in heavily contaminated specimens of sputum; (4) that in a comparison in which 170 tuberculous specimens were treated by the Teepol and the lauryl sulphate method, the number of positive cultures was slightly higher and the number of contaminated cultures was slightly lower with the newer method; and (5) that only 10 of 1,500 specimens grew contaminants after 24 hours incubation when the mixture was allowed to act for 30 minutes but when this period was extended to 60 minutes the number of contaminants was reduced to 2.

In the recommended method the mixture of lauryl sulphate and alkali is kept at 37°C. and when required 3 ml. are added to 1 ml. of the specimen and shaken in a Kahn shaker for 30 minutes. The mixture is then neutralized with dilute sulphuric acid containing bromocresol purple, and centrifuged at 3,000 r.p.m. for 30 minutes.

S. R. M. Bushby

BELKNAP, Della R., CAMIEN, M. N. & DUNN, M. S. **Amino Acid Composition of Mycobacterial Cell Wall.** *Proc. Soc. Exper. Biol. & Med.* 1961, June, v. 107, No. 2, 289–92, 2 figs.

BRAUNSTEIN, H. & ADRIANO, S. M. **Fluorescent Stain for Tubercle Bacilli in Histologic Sections. I. Diagnostic Efficiency in Granulomatous Lesions of Lymph Nodes.** *Amer. J. Clin. Path.* 1961, July, v. 36, No. 1, 37–40. [11 refs.]

"1. Sections from 66 examples of granulomatous inflammation in lymph nodes were examined for tubercle bacilli by means of a fluorescent stain technic.

"2. In 33 cases (50%) positive results were obtained. This contrasted with only 9 positive results with conventional acid-fast stains (14%).

"3. In 13 of 17 instances in which culture was performed, positive results were obtained; in 2 of the 4 with negative results, the fluorescent dye technic demonstrated organisms.

"4. In 14 examples the diagnosis was established only by the fluorescent dye technic.

"5. The superior sensitivity of the fluorescent dye technic would seem to justify its substitution for conventional staining methods in the examination of tissue sections for acid-fast bacilli."

TARSHIS, M. S. **Further Investigation on the Usefulness of the Niacin Test for distinguishing Human Tubercle Bacilli from other Mycobacteria. I. A Comparative Study of Three Direct Qualitative Micro-Methods, using Fresh Cultures.** *Amer. J. Clin. Path.* 1961, May, v. 35, No. 5, 461–7, 1 fig. [Reprinted from *Tech. Bull. Registry Med. Technol.* 1961, Apr., v. 31, No. 4, 59–65.]

In these 3 methods, aqueous extracts of the bacillary growth were obtained by flooding 7, 10, 14 and 21 days' growth on the Tween-albumin niacin test medium [this *Bulletin*, 1960, v. 35, 1227; 1961, v. 36, 485] with 10 drops of distilled water, removing the water after 5 minutes and autoclaving it. 2 drops of the extract were placed in 3 depressions in glazed porcelain plates and then to one was added 1 drop of 1·5% *o*-tolidine in 95% ethanol, to another 1 drop of 4% aniline in 95% ethanol, and to the third 1 drop of 3% benzidine in absolute ethanol. To each was then added 1 drop of 10% cyanogen bromide. The results were read 5 minutes after mixing, positive results being shown by coral red, yellow and violet-pink colours, respectively, in each of the 3 methods.

50 human strains, 2 bovine and 2 avian strains of tubercle bacilli and 25 unclassified mycobacteria and 5 saprophytes were chosen for this study. All the human tubercle bacilli gave positive reactions with all 3 methods, whereas all the other strains, except 2 gave either doubtful or negative ones. The Copenhagen and Ravenel bovine strains gave slightly positive results with each method after the 10th day. The strength of the positive reactions increased with incubation, and with the earlier cultures the *o*-tolidine cyanogen was slightly less sensitive than the others.

All the reagents should be stored at 5°C., and when kept in tightly sealed coloured bottles they may be used for up to 10 weeks. The tests should be done under a well ventilated bacteriological hood, and several drops of 10% ammonia should be added to each test to destroy the cyanogen bromide.

S. R. M. Bushby

TARSHIS, M. S. **Further Investigation on the Usefulness of Niacin Test for differentiating Human Tubercle Bacilli from other Mycobacteria. II. A Comparative Study of Three Direct Qualitative Micro-Methods using Stored Cultures of Varying Ages.** *Tubercle.* 1961, Mar., v. 42, No. 1, 101–6. [14 refs.]

Extracts of the cultures on Löwenstein-Jensen medium or Tween-albumin niacin test medium [this *Bulletin*, 1960, v. 35, 1227] were obtained by flooding the culture with 2–10 drops of distilled water, and after autoclaving at 15 lb. per square inch for 15 minutes they were tested by the 3 recently described methods [*ibid.*, 1961, v. 36, 485]; the principal difference between the methods was in the aromatic reagent. The reagents were 1·5% *o*-tolidine, 4% aniline and 3% benzidine, each being in 95% ethanol, and they were added to the 2 drops of extract in the depressions in white, glazed porcelain spot plates. To each was then added 1 drop of 10% cyanogen bromide.

The results were the same with each method, irrespective of whether the culture was 2 weeks old or 2½ years old. 6 human strains, including 1 streptomycin-, 1 PAS- and 2 isoniazid-resistant strains, gave positive results, but both the bovine strains (Copenhagen and Ravenel) gave slightly positive reactions, as did 2 Battey strains when their cultures had been stored for 60–120 days. The 2 avian strains, 2 unclassified photochromogens and 2 scotochromogens gave completely negative reactions, but 2 saprophytic mycobacteria gave doubtful results with the stored cultures.

The importance of using freshly prepared reagents is emphasized.

S. R. M. Bushby

TARSHIS, M. S. **Some Useful Procedures for Group Differentiation of *Mycobacterium tuberculosis*, Unclassified Mycobacteria, and Saprophytic Acid-Fast Bacilli.** *J. Lab. & Clin. Med.* 1961, Mar., v. 57, No. 3, 480–89, 2 figs. [29 refs.]

The investigations were done on 50 freshly isolated human strains, 2 bovine strains and 2 strains of avian tubercle bacilli, 50 strains of unclassified mycobacteria and 5 strains of saprophytic acid-fast bacilli.

In most instances the positive peroxidase and neutral red tests and the ability to grow as cords enabled the human and bovine tubercle bacilli to be distinguished from the avian tubercle bacilli, the unclassified mycobacteria and the saprophytes; however, only the positive peroxidase and neutral red tests enabled some of the Battey strains to be differentiated from the other unclassified bacilli, the avian tubercle bacilli and the saprophytes. Inability of the tubercle bacilli to grow in Brewer thioglycollate medium distinguished these strains from most of the other mycobacteria. Except for one of the strains of bovine tubercle bacilli, the niacin test was positive only with human strains of tubercle bacilli. True cording was seen only with the tubercle bacilli, although some of the other strains showed pseudocording. The neutral red test used in conjunction with the cording test is valuable for determining virulence.

S. R. M. Bushby

TAKAHASHI, Y. & YAMAMOTO, K. Sur l'antigène commun entre le bacille tuberculeux et le bacille de la lèpre. **[The Common Antigen of the Tubercle Bacillus and the Leprosy Bacillus]** *C.R. Soc. Biol.* 1960, v. 154, No. 11, 2160–63, 1 fig.

A comparison of the antibodies present in the sera of 201 tuberculous patients and 50 leprosy patients, negative to tuberculin, showed that 100% and 86%, respectively, of these 2 groups contained antibodies

detectable with cells sensitized with the polysaccharides from tubercle bacilli, whereas only 55% and 4%, respectively, contained antibodies detectable with the proteins of this bacillus, and 80% and 44%, respectively, contained antibodies to its phosphatides.

The authors interpret these findings as supporting the contention that of these 3 antigens of the tubercle bacillus, the polysaccharide is the one most closely related to that of the leprosy bacillus, and that this finding contrasts with that found for the atypical bacilli, in which the protein antigen is most closely related. These results, therefore, correlate with the contention that there is cross immunity but not hypersensitivity in tuberculosis and leprosy.

S. R. M. Bushby

DEVIGNAT, R. **Multiplication of Hansen's Bacillus in Complex Symbiosis *in vitro*.** [Correspondence.] *Nature*. 1961, May 27, v. 190, 832.

[An abstract of this paper appeared in the *Tropical Diseases Bulletin*, 1961, v. 58, 1247.]

YOUATT, Jean. **Pigments produced by *Mycobacteria* on Exposure to Isoniazid.** *Australian J. Exper. Biol. & Med. Sci.* 1961, Feb., v. 39, Pt. 1, 93–100, 7 figs.

BCG, but not its isoniazid-resistant variants, produces soluble pigments when shaken with isoniazid [this *Bulletin*, 1959, v. 34, 380], and the present paper is concerned with the methods for producing and purifying these pigments.

The BCG in concentrations of 20–25 mgm. per ml., were suspended in 0·01 M phosphate buffer containing 200 μgm. per ml. of isoniazid and ammonium chloride per ml., and shaken at 37°C. for 24–30 hours at 90 cycles per minute. At the end of this time the supernatant fluid was collected and replaced with the same suspending fluid for another 24 hours, after which it was again collected. The pigments were then isolated by chromatography, with either Whatman 3 or 3MM paper or carboxymethylcellulose.

Oxygen was found to be essential for pigment production, and the intensity of the soluble pigment increased with increased concentrations of isoniazid up to 200 μgm. per ml. and of ammonium chloride up to 100 μgm. per ml.; calcium chloride had a comparable effect, but sodium chloride, potassium chloride, ammonium sulphate, ammonium citrate, sodium sulphate, sodium phosphate (dibasic), sodium acetate, potassium phosphate (monobasic), magnesium sulphate, manganese sulphate, ferrous sulphate and zinc sulphate were ineffective. The presence of sodium citrate, sodium acetate, glucose, glycerol, glycine, serine, adenine, uracil, asparagine or casein hydrolysate did not increase the production. Sodium cyanide, sodium azide, 2:4-dinitrophenol [*ibid.*, 1959, v. 34, 279] and 3 hydrazides [*ibid.*, 1961, v. 36, 185], substances known to inhibit the uptake of isoniazid, inhibited production of the pigments. The chelating compounds, oxine, versene and *o*-phenanthroline had no effect. At *p*H 6, pigment production was reduced and at *p*H 8, more coloured bands were pro duced in the chromatograms.

4 compounds were regularly produced and all wer examined spectroscopically and one was fairly exter sively examined by chemical methods, but no definit conclusion could be drawn about its identity. It wa a small molecule with 1 or more carbonyl groups; was weakly basic and it could have been a Schiff bas Another of the pigments may have been riboflavin

From the results the author suggests that the drug i taken up by the cells, where it combines with certai carbonyl compounds which are essential intermediate in the cell metabolism. These hydrazones are broke down to yield 4-pyridine aldehyde and Schiff bases c the carbonyl compounds. *S. R. M. Bushby*

TARSHIS, M. S. **The Preservation of Mycobacteria b Freezing in Various Diluents.** *Amer. Rev Respiratory Dis.* 1961, May, v. 83, No. 5, 762–6 [Refs. in footnotes.]

The note describes the results of storing, at −20°C for 3 years, 2 strains each of the human, bovine an avian varieties of the tubercle bacilli, 6 unclassifie mycobacteria, 2 saprophytic mycobacteria and 10 drug resistant strains of human tubercle bacilli. The bacill were suspended in five different fluids:—distilled water 0·01% charcoal in distilled water, 0·85% NaCl in dis tilled water, 0·1% bovine albumin fraction V in distille water, and 5% glycerol broth. At yearly intervals th suspensions were thawed and examined for changes i staining reaction, viability and drug resistance, an tested by the thioglycollate, peroxidase, neutral red cording and niacin tests; each suspension was thu twice thawed and re-frozen.

Apart from some light staining and pleomorphi bacilli in smears of one of the avian strains preserve in the saline, albumin and glycerol broth diluents, an also an increase in the colour of the photochromoger no change was found in the various strains; after years' storage the strains did, however, take a littl longer to grow. The author therefore claims that thi method of preserving these cultures is simple, safe an economical and that it requires little material, time equipment or storage space. *S. R. M. Bushby*

WALLACE, J. G., MITCHISON, D. A., REES, R. J. W BHATIA, A. L. & GANGADHARAM, P. R. J. **Th Virulence of South Indian Tubercle Bacilli i Mice and Guinea-Pigs infected by the Intra venous Route.** *Tubercle*. 1961, June, v. 42, No 2, 212–17. [21 refs.]

This comparison of virulence was made between strains from tubercle bacilli isolated from patients i India and 6 strains from patients in England; all th strains were sensitive to isoniazid and the animal test were done in London, Porton (England) and Madra Mice were infected intravenously with 0·1 mgm. an 0·025 mgm. of culture and guineapigs, intramuscularly with 1·0 mgm. Assessment of virulence was based o the extent of tuberculosis in the guineapig and th

proportion of mice dying with the disease and the average survival time.

The principal finding of the study was that although there was a well marked difference in the guineapig between the virulence of the British strains and the Indian strains, the indices ranging from 0·08 to 0·58 with the Indian strains and 0·78 to 2·14 for the British strains, in the mouse, especially with the larger inoculum, no difference was found. (With the smaller inoculum the average survival time of the mice infected with the Indian strains may have been a little longer.)

Two of the British strains and 2 of the Indian strains were also inoculated intravenously into guineapigs, and at intervals the numbers of viable bacilli present in the spleen were determined. One of the Indian strains was definitely attenuated and although the number of viable bacilli with this strain increased during the first 14 days, it then decreased; with the other more virulent strains the numbers progressively increased. The failure of the mouse test to detect the difference in virulence is therefore attributed to the size of the inoculum which was sufficient to induce lethal effects before immunity began to produce an effect against the attenuated strains.

[See also this *Bulletin*, 1960, v. 35, 695.]

S. R. M. Bushby

FURNISS, A. L., COLLINS, C. H. & MARKS, J. **A Case of Infection with Avian Type Tubercle Bacilli.** *Monthly Bull. Ministry of Health & Pub. Health Lab. Service (directed by Med. Res. Council).* 1961, July, v. 20, 126–7.

This was a case of achalasia of the cardia complicated by pulmonary tuberculosis which was diagnosed in 1946 when the patient was aged 54 years; she was treated periodically with streptomycin and PAS. In 1958 she was admitted to hospital after several haemoptyses, and acid-fast bacilli with unusual characteristics were repeatedly grown from her sputum. One culture inoculated into a guineapig failed to produce tuberculosis. She died in 1960 after a series of haemoptyses and no post-mortem examination was made.

Shortly before death an acid-fast bacillus was isolated and this was examined more extensively than those previously isolated. The growth on Löwenstein-Jensen medium was smooth, buff-coloured and emulsified readily; pigmentation was unaffected by light. Growth occurred as well at 44·5°C. as at 37°C.; it was slight at 25°C. and none occurred on nutrient agar. The strain was resistant to the antituberculous drugs, except cycloserine; it was also highly resistant to tri-phenyl tetrazolium. Catalase activity was only moderate.

The strain was pathogenic to fowls, but not to guineapigs; it produced minimal lesions in the main viscera in rabbits; the bacilli were, however, readily recoverable from the liver and spleen.

[See also this *Bulletin*, 1946, v. 21, 225.]

S. R. M. Bushby

WALLACE, J. G. **The Heat Resistance of Tubercle Bacilli in the Lungs of Infected Mice.** *Amer. Rev. Respiratory Dis.* 1961, June, v. 83, No. 6, 866–71, 4 figs.

The purpose of the experiments described in this paper was to test the hypothesis of REES and HART [this *Bulletin*, 1961, v. 36, 594] that the constant level of bacillary counts in mice with chronic tuberculosis is due to the organisms being in a static non-multiplying state. According to WINSLOW and WALKER (*Bact. Reviews*, 1939, v. 3, 147), the resistance of bacteria to heat depends on the metabolic activity and speed of cell division and so the thermal resistance of tubercle bacilli in the lungs of chronically infected mice was compared with that of bacilli in lungs from acutely infected mice, from actively growing cultures and from stationary cultures. Preliminary experiments with H37Rv showed that the killing rate was too slow at 50°C. and too rapid at 55°C., and so 53°C. was chosen as the optimum temperature. The suspensions were exposed for periods up to 120 minutes, and viable counts were made at appropriate intervals.

The results showed that the tubercle bacilli from an old culture were decidedly more resistant than those from a young culture and that the bacilli from the chronically infected mice behaved like those from the old culture. Those from the mice with acute disease were more susceptible to heat but the difference between them and those from the chronic infection was small. The results of these experiments, therefore, support the static hypothesis. *S. R. M. Bushby*

TAKAHASHI, Y., KUREMATSU, S., YAMAMOTO, K. & SASAKI, A. Sur l'hétérogénéité sérologique des antigènes tuberculeux aptes à être considérés comme sérologiquement simples. [**The Serological Heterogeneity of the Tuberculous Antigens considered to be Serologically Pure**] *C.R. Soc. Biol.* 1960, v. 154, No. 11, 2163–6.

Rabbits were injected with 10 mgm. of 5 different fractions of tubercle bacilli, the fractions being suspended in an adjuvant consisting of water-arlacel-drackeol plus a non-antigenic wax from BCG. At intervals up to 10 weeks the animals were bled and their sera tested against red cells sensitized with tuberculo-protein, -polysaccharide and -phosphatide. The 5 fractions were:— polysaccharide S4, isolated with urea and deproteinized by the method of Sevag; polysaccharide TS-2, obtained by precipitation with methanol from a Sauton tuberculin deproteinized with trichloracetic acid; protein TR-1b, isolated from Sauton tuberculin by means of trichloracetic acid and therefore comparable to PPD; protein R-12ab, isolated by a similar process to TR-1b; and the phosphatide Pd(n)1.

The results showed that only the rabbit injected with TR-1b reacted strongly to tuberculin, and that this animal developed high-titre antibodies to all three antigens. The animals injected with TS-2 and R-12ab reacted weakly to tuberculin and they also developed antibodies but the titres, especially in the rabbit given R-12ab, were relatively low. Although neither of the rabbits injected with S4 and Pd(n)1 reacted to tuberculin, the one given S4 developed antibodies to all 3 antigens, and the other, receiving the phosphatide antigen, developed antibodies to only this antigen; the antibodies to the protein and polysaccharide antigens

in the rabbit injected with S4 were, however, of low titre.

Thus only the phosphatide antigen would appear to have been pure, and the fraction TR-1b, corresponding to PPD, was apparently very impure. The results with fraction TS-2 indicate that deproteinization with trichloracetic acid does not remove all the tuberculoprotein ; after treatment by the Sevag method this loses all its tuberculin properties. *S. R. M. Bushby*

TAKAHASHI, Y., FUJITA, S. & SASAKI, A. **The Specificity of the Passive Hemagglutination Methods used in Serology of Tuberculosis.** *J. Exper. Med.* 1961, June 1, v. 113, No. 6, 1141–54. [35 refs.]

This paper presents in greater detail the results previously reported (*C.R. Soc. Biol.*, 1960, v. 154, 692). The methods of preparing the protein, polysaccharide and phosphatide antigens are described. More than 20 of these fractions were prepared but as some behaved so similarly, the results with only 11 are described. The sera of rabbits immunized with heat-killed H37Rv were used as the source of antibodies.

The results obtained with the polysaccharide antigens in the Middlebrook-Dubos test (MD test) and with the protein antigens in the Boyden test (B test), in which the red cells are treated with tannic acid, showed that the polysaccharide fraction did not sensitize cells for the B test unless they contained more than 1% nitrogen and that the protein fractions containing less than 1% polysaccharide did not sensitize cells for the MD test; antigen mixtures in which the minor antigen is present to the extent of 1% or more thus act as dual antigens.

These results support the contention that agglutination in the MD test is due to sensitization with the polysaccharide antigen and in the B test, to the protein antigen. Absorption tests with sensitized cells and antigens adsorbed on to kaolin, confirmed this contention. Absorption tests with the sensitized kaolin, also showed that the anti-phosphatide antibody is distinct from the anti-protein and anti-polysaccharide antibodies, and by appropriate absorption tests the 3 antibodies were obtained separately. 2 of the polysaccharide and 1 of the protein antigens failed to sensitize cells, in spite of their giving a positive precipitin reaction, indicating that haemosensitizing ability and antigenicity are distinct properties. *S. R. M. Bushby*

TURK, J. L. **Active Sensitization to Tuberculin with Cells from Tuberculin-Sensitized Guinea Pigs.** *Nature.* 1961, Mar. 18, v. 189, 889–90.

Hypersensitivity to tuberculin, lasting for 3 months or more can be passively transferred to non-sensitized guineapigs by injecting them with live leucocytes from sensitized animals ; the experiments described in the present paper show that the transfer of hypersensitivity is probably due to active sensitization of the recipients superimposed on the passive hypersensitivity transferred with the donor cells, rather than due to the survival of donor cells.

Guineapigs were actively sensitized with heat-killed tubercle bacilli, and preparations of some of the cell from the spleen, lymph nodes and peripheral blood some of the cells were disrupted by freezing and thaw ing in the presence of deoxyribonuclease. Live cel preparations were injected intravenously and the dis rupted preparations were injected intradermally int non-sensitized guineapigs. The animals were then teste periodically with intradermal PPD.

In the animals given the live cells, sensitivity t tuberculin was detected from the day of transfusio and for at least 3 months. However, as skin graft from the donor guineapigs transplanted into groups o 13 of the recipient animals on the 9th and 28th day were rejected by 10 and 12, respectively, of each group the tuberculin state was unlikely to be due to surviva of the transferred cells. In the guineapigs that receive the disrupted cells, weak hypersensitivity to tuberculi developed 7–14 days later but it was stronger whe re-tested after 17 days. Control animals given no cell or disrupted cells from non-sensitized animals, showe little or no hypersensitivity to tuberculin although som of the animals reacted weakly after repeated testin with PPD.

The author therefore concludes that transient delaye hypersensitivity is transferred by intact leucocytes and to a lesser extent, by spleen and lymph node cells, an that it may be followed by superimposed active immuni zation which persists far beyond the time when th donor cells would have been destroyed by a homograf reaction. A further property of these cells is thei ability to enhance the immune response against multipl injection of PPD. *S. R. M. Bushby*

STEENKEN, W., Jr. **The Persistence of Tubercle Bacilli in Caseous Lesions in the Experimental Animal (Guinea Pig).** *Amer. Rev. Respiratory Dis.* 1961, Apr., v. 83, No. 4, 550–54, 2 figs. [17 refs.]

The purpose of these experiments was to determin how long heat-killed tubercle bacilli remain morpho logically intact and stainable in caseous lesions i guineapigs, and to discover how long living, avirulen tubercle bacilli, under similar conditions, remain stain able and culturable by conventional techniques.

Heat-killed H37Rv and living H37Ra were inoculate into the right testicle, right groin and intraperitoneall in male guineapigs, and into the right groin and intra peritoneally in female guineapigs. Three males an females of each group were killed at intervals of 2 months and smears were made from each of the caseou lesions and cultures from those of the animals injecte with the avirulent strain. The numbers of dead H37R and viable H37Ra fell during the first 10 months, t reach a plateau, at which they remained till the 28t month, when the experiment was ended. These effect were confirmed in a second experiment, in which th same procedure was followed except that greater car was taken to give equal numbers of bacilli in th inocula ; this experiment ran for 36 months. Tuber culin tests showed that all the animals developed sensi tivity, but they reacted less strongly at the end of th experiment.

Both heat-killed and viable attenuated bacilli have, therefore, been shown to persist in caseous lesions for up to 36 months. *S. R. M. Bushby*

HALPERN, B. N., STIFFEL, C., BIOZZI, G. & MOUTON, D. Influence des hormones sexuelles sur la stimulation de la fonction phagocytaire du système réticulo-endothélial provoquée par l'inoculation du bacille de Calmette-Guérin (BCG) chez la souris. **[The Influence of Sex Hormones on the Stimulation of Phagocytic Function of the Reticulo-Endothelial System Provoked by Inoculation of BCG in the Mouse]** *C.R. Soc. Biol.* 1960, v. 154, No. 11, 1994–2001, 2 figs. [10 refs.]

The rate of clearance of colloidal carbon after intravenous injection and the relative weight of the liver and spleen to the total body weight were used as a measure of the phagocytic activity of the reticulo-endothelial system. The experiments were done with mice weighing 20–25 gm., which were injected intravenously with 40 μgm. BCG, given to castrated animals 8 days after removal of the gonads and to those given hormone therapy, 4 days after the first dose.

The results of the first of the experiments which were done in intact animals and with no hormonal therapy showed that the increase in phagocytic activity induced by BCG was higher in the female during the period 11th–20th days than in the male. The results of the other experiments which included castrated animals with and without treatment with testosterone, showed that castration of the male did not affect the increase of phagocytosis by BCG, but the administration of the oestrogen αα-dimethyl-β-ethyl methoxy allenolic acid (Novestrine) to both normal and castrated males increased this activity; in the normal and castrated female administration of testosterone had no effect.

Thus, these experiments show that although there is little difference between the phagocytic power of normal male and female mice, the activity after stimulation by BCG is higher in the female and that this difference is due to the presence of oestrogens and not to a depressing effect of androgens. These observations are therefore of importance with regard to the difference in response of the sexes to infections and malignant tumours. *S. R. M. Bushby*

FONG, J., CHIN, D. & ELBERG, S. S. **Studies on Tubercle Bacillus-Monocyte Relationship. IV. Effects of Passage in Normal and Immune Systems upon Virulent Bacilli.** *J. Exper. Med.* 1961, July 1, v. 114, No. 1, 75–87. [15 refs.]

This paper reports a decrease in the virulence of H37Rv to mice when passaged through cultures of normal monocytes or immune monocytes. The monocytes were from the peritoneal cavity of normal rabbits and rabbits immunized with BCG, the exudate being induced by the injection of klearol, and the cells were grown in Tyrode solution containing normal or immune rabbit serum. The infected cell cultures were incubated for 6 days, at the end of which time the bacilli were released from the cells by lysing the cells with saponin. The virulence tests were done by noting the survival time of mice injected intravenously with the bacillary suspensions.

Comparison of the virulence of the bacilli before and after growth in the immune and normal monocytes showed that growth in the cells caused a decrease in virulence, in spite of the infecting dose of the passaged bacilli being twice as large as that of the unpassaged bacilli. This reduction of virulence was apparently the result of a modification to the bacilli occurring in the intracellular environment for no change in virulence occurred in bacilli exposed to the constituents of the tissue culture system (immune serum, immune cell lysate, saponin and salt solution). Growth of immune-passaged bacilli in normal laboratory media restored the virulence. Neutral red tests on the bacilli passaged through the immune and normal cells showed that they had lost the power to bind the dye, suggesting a change in the cell surface, and this suggestion was supported by the observation that the passaged bacilli were very much more sensitive to the lethal effects of sodium oleate and monocyte lysate; the passaged bacilli showed, however, an increased resistance to streptomycin.

The discrepancy between this lowering of virulence and the increase that occurs in bacilli from lungs of infected mice, could be due to a selective action occurring *in vivo* [this *Bulletin*, 1957, v. 32, 800].

[For references to previous parts, see this *Bulletin*, 1959, v. 34, 1170.] *S. R. M. Bushby*

SUTER, E. **Passive Transfer of Acquired Resistance to Infection with *Mycobacterium tuberculosis* by means of Cells.** *Amer. Rev. Respiratory Dis.* 1961, Apr., v. 83, No. 4, 535–43, 1 fig. [28 refs.]

In the first series of experiments described in this paper, an *in vitro* system was used for exploring the possibility of detecting a factor that is transferable from guineapigs vaccinated with BCG. 3–5 weeks after vaccination, guineapigs were injected intraperitoneally with glycogen, and 4 days later the cells in the peritoneal exudate were collected and injected intraperitoneally into non-vaccinated guineapigs. Cells from the recipient guineapigs were collected from the peritoneum 4 days later and cultured *in vitro* with R1Rv. In another of these experiments, cells from lymph nodes of immunized guineapigs were injected intracardially into recipient guineapigs and peritoneal exudate cells were again collected 4 days later and similar cultures were made from them. Counts of the average number of intracellular tubercle bacilli present at 1, 5 and 8 days showed that the multiplication of the bacilli in the mononuclear exudate of the animals that had received cells from vaccinated animals was approximately 50% less than in those that had received cells from non-vaccinated animals. Experiments with mice gave similar results.

In the second series of experiments, splenic and lymph node cells from vaccinated and non-vaccinated guineapigs were injected into non-vaccinated guineapigs and the cell transfers made repeated on the 4th, 9th,

12th and 16th days. On the 5th day of the experiment the recipients were injected intradermally with four 10-fold increasing dilutions of BCG, and measurement of the taken lesion from 1 to 61 days after injection showed that the lesions were smaller in the animals that had received cells from vaccinated donor animals. There was no indication of a Koch phenomenon in the animals that received the cells from vaccinated animals.

In the third series of experiments, donor cells from spleens of vaccinated and non-vaccinated mice were injected intraperitoneally at intervals into normal mice, and on the day of the second transfer the recipients were injected intravenously with R1Rv. 4 weeks later the number of bacilli present in the lungs, the histological appearance of the lungs and the weight of the spleens were compared with those of mice that had been immunized with BCG. The results showed that whereas the transfer of cells from immunized donor mice conferred some protection, it was less than that afforded by direct vaccination with BCG, and that 4 injections of donor cells were more effective than 2 injections. In the last experiment, in which mice were given a virulent infection with the Vallée bovine strain, injection of cells from immunized donor mice, however, afforded no protection, but direct immunization with BCG afforded very definite protection.

The author concludes that although the transfer of cells from immunized animals affords some protection against alternated infection, it affords little protection against large doses of virulent bacilli.

S. R. M. Bushby

MILLMAN, I. **Nonspecific Resistance to Tuberculosis.** *Amer. Rev. Respiratory Dis.* 1961, May, v. 83, No. 5, 668–75. [23 refs.]

The experiments described in this paper were regarded as preliminary steps in investigating the specificity of the protection afforded by fractions of tubercle bacilli, especially in view of the big difference between the minimal amounts of live vaccines and dead vaccines or extracts necessary to give protection. Groups of mice were vaccinated intraperitoneally with graded doses of various immunizing agents, and they were subsequently challenged with living tubercle bacilli, pneumococci, salmonellae or *Klebsiella pneumoniae*; protection was assessed by the prolongation of the survival time.

In the experiments in which the challenge was with tubercle bacilli, 1·0 mgm. of residue from methanol-extracted bacilli gave protection equal to that of 0·001 mgm. of lyophilized vaccine; heated for 30 minutes at 100°C., had, however, little protective power. The methanol extract, extracted in the cold, afforded no protection, but that extracted at 65°C. was highly protective. This active fraction was subdivided into a white fraction which separated out when the extract cooled, and a tan fraction which was the more active. Fractionation of these 2 fractions by chromatographic separation on a magnesium-silicate-celite column, yielded several active components, but the sum of all their activities was less than that of lyophilized vaccine. Waxes C and D and Anderson's purified wax afforded no protection.

Zymosan and the lipopolysaccharides of *Escherich coli* and salmonellae were protective against the tuberc bacillus, but they were somewhat toxic, and methan extracts of *Escherichia coli*, made at 25°C. and 65°C also afforded some protection. Neither glycogen n polysaccharide from *Pseudomonas pyocyanea* w active. Injection of the white and brown methano extract fractions of the tubercle bacillus afforded so protection against challenge 2–4 weeks later wi *Salmonella enteritidis, Salm. typhi-murium, Strept coccus pneumoniae* and *K. pneumoniae*. Injection denatured globulin (horse serum), which stimulates t phagocytic cells of the reticulo-endothelial syste afforded no protection against challenge with t tubercle bacillus. In spite of this apparent discrepanc however, the author finds it tempting to assume th the protection afforded by the methanol extracts of t tubercle bacillus is non-specific. *S. R. M. Bushby*

OSHIMA, S., MYRVIK, Q. N. & LEAKE, Eva. **The Demo stration of Lysozyme as a Dominant Tubercul static Factor in Extracts of Granulomatou Lungs.** *Brit. J. Exper. Path.* 1961, Apr., v. 4 No. 2, 138–44, 3 figs. [17 refs.]

The experimental conditions described in this repo were intended to reproduce the cellular milieu of t stages of re-infection tuberculosis and thus offer means for studying the intracellular and extracellul biochemical environment associated with this cellul response. A diffuse granulomatous lesion was induce by injecting intravenously 5 mgm. of BCG into rabbit which had been vaccinated 6 weeks earlier with 5 mgm. heat-killed BCG suspended in Freund adjuvan 4 days after the injection of the living BCG the lun of the rabbits were homogenized with 2 volumes water and the *p*H value of the homogenate was adjust to 3·0. The homogenate was kept at 4°C. overnigh the *p*H value was readjusted to 7·0, and the extract w collected by centrifuging. Lungs from normal rabbi from vaccinated but unchallenged rabbits and fro unvaccinated and unchallenged rabbits were treate similarly and the extracts collected.

The smallest quantities of the extracts that inhibite the growth of *Mycobacterium phlei* and *Myco. bov* were determined in Dubos medium, and the lysozym content of the extracts was determined against *Sarci lutea*. Similar determinations were also made on fra tions of the extracts obtained by column chromat graphy on cation exchange cellulose.

The results show that the tuberculostatic power the extracts from the vaccinated and challenge animals was 4–8 times higher than those from t lungs of normal rabbits, vaccinated rabbits or cha lenged but unvaccinated rabbits. The chromatograph analysis showed that this activity was mainly due lysozyme but that there was a second factor, repr senting approximately 20% of the activity, that w more active against *Myco. bovis* than *Myco. phle* further investigations are being made to characteri this second factor. The present findings are regarde as demonstrating that the granulomatous respon produces an environment inimical to tubercle bacil

S. R. M. Bushby

ACHESON, R. M., MYNORS, Lavinia & TAYLOR, K. B. **Cortisone and Tuberculin as Adjuvants to Streptomycin and Isoniazid in the Chemotherapy of Experimental Tuberculosis in the Guinea Pig.** *Amer. Rev. Respiratory Dis.* 1961, May, v. 83, No. 5, 753–6, 2 figs. [13 refs.]

Guineapigs were vaccinated with the vole bacillus, and 3 weeks later, when 91 of the 96 animals reacted to 1:100 OT, 85 of the sensitized animals were infected with 0·01 mgm. of virulent bovine bacilli. These infected animals were then divided into 4 groups. Three groups were treated daily with 5 mgm. streptomycin and 2 mgm. isoniazid, but in addition to this treatment 1 of these groups also received daily doses of 2·5 mgm. cortisone, and another received tuberculin, the daily dose increasing weekly for 6 weeks from 0·0075 mgm. to 0·045 mgm.; the fourth group received no treatment. The effects of these treatments were assessed by the extent and spread of the disease.

The results support the contention that cortisone lessens haematogenous spread of the disease, for although all the treated animals showed less extensive tuberculosis than those receiving no treatment, the mean infection index was only 3·8 for the group that received this steroid as compared with 102·2 for the untreated group, 7·8 for the group that received no adjuvant treatment and 6·1 for the group that received the tuberculin. The incidence of abscesses at the vaccination site was least in the animals that received the tuberculin. *S. R. M. Bushby*

TAI, F. H., JEN, T. M., CHU, B. Y. & HAN, S. H. **Isoniazid Prophylaxis of Tuberculous Infection in Mice.** *Amer. Rev. Respiratory Dis.* 1961, May, v. 83, No. 5, 711–17. [21 refs.]

In this study 4 groups of 600 mice were given 3 mgm. isoniazid per kgm. in the drinking water each day for 4–5 weeks, and at the end of this period the animals of two of the groups and those of an untreated control group were infected intraperitoneally with 1·5 mgm., wet weight, H37Rv. Treatment with isoniazid was continued in 1 of the infected treated groups and in 1 of the uninfected treated groups for another 10 weeks, after which all of the surviving mice of the treated groups were infected with H37Rv (2 of these groups, 1 that had been treated for 4–5 weeks and the other for 14–15 weeks, were thus re-infected). At the time of this re-infection a 6th group, to serve as controls, was also infected.

Comparison of the numbers of mice surviving 14 weeks after the date of the second infection showed that the pretreatment with isoniazid had exerted a prophylactic effect. The percentage mortality of the group that was re-inoculated after 4–5 weeks' pretreatment was 25·2 and of the group that received a further 10 weeks' treatment before re-challenge was 17·3, these percentages contrasting with 68·3 for the control group that had received no treatment. That this was prolonged prophylactic effect was shown by the mortality of the group that received no treatment for the 10 weeks before challenge being 19·3% as compared with 18·8% for the group that received 14½ weeks' treatment before challenge and 53·9% for the untreated group.

Histological examination of the organs of some of the animals of each group confirmed the prophylactic effect of the treatment, for the disease was less in all of the treated animals. Sensitivity tests made of cultures from 18 of the treated animals showed the tubercle bacilli to be still sensitive to isoniazid.

S. R. M. Bushby

KUBICA, G. P. & VESTAL, Annie L. **A Rapid Drug-Susceptibility Test for Mycobacteria. Preliminary Report.** *Amer. Rev. Respiratory Dis.* 1961, Apr., v. 83, No. 4, 593–5. [Refs. in footnotes.]

This method is a modification of the method described by PITAL *et al.* [this *Bulletin*, 1959, v. 34, 164] and SORENSEN, R. H. (*Med. Technicians Bull.* Supp. to U.S. Armed Forces Med. J., 1959, v. 10, 144), To various concentrations of the drugs in 0·1 ml. distilled water is added 1 ml. of a suspension of the test organism that was just turbid. After 1 or 2 days' incubation at 37°C., 1 drop of 0·05% solution of resazurin is added, and the tubes are re-incubated. As soon as the control tube containing no drug turns pink, the drug-containing tubes are read; a pink or red colour is interpreted as indicating resistance and a blue tint as susceptibility to the drug. With rapidly growing mycobacteria the dye should be added at the same time as the bacilli.

In a comparison of this rapid method with one of the routine drug-susceptibility procedures, good correlation was found with a variety of mycobacteria tested against isoniazid, PAS and streptomycin. There were, however, two exceptions, in which the strains appeared more resistant to isoniazid by the rapid method. This discrepancy may have been due to the delayed effect of isoniazid, and if this is confirmed then the incubation period of the drug and the bacilli before the addition of resazurin may have to be extended to 4 days. *S. R. M. Bushby*

CANETTI, G. & GROSSET, J. Teneur des souches sauvages de *Mycobacterium tuberculosis* en variants résistants à l'isoniazide et en variants résistants à la streptomycine sur milieu de Loewenstein-Jensen. [**Frequency of Isoniazid-Resistant Variants and Streptomycin-Resistant Variants in Wild Strains of *Mycobacterium tuberculosis* on Löwenstein-Jensen Medium**] *Ann. Inst. Pasteur.* 1961, July, v. 101, No. 1, 29–46, 1 fig. [16 refs.]

This study was made on 22 strains of tubercle bacilli from patients who had not been treated with antituberculous drugs. Inocula from cultures of each of these strains growing in Dubos medium were placed on Löwenstein-Jensen medium to which had been added graded amounts of streptomycin and isoniazid and the number of colonies growing were counted after 25 and 40 days' incubation.

The number of colonies growing after 40 days' incubation from an inoculum of 10^6 bacilli in the presence of 0·05 μgm. isoniazid per ml. varied considerably from strain to strain, the lowest being 39 and the highest 40,000. The variation in the presence of 0·1 μgm. per ml. was much less, only 2 of the strains growing more than 100 colonies (110 and 180), and at a concentration of 1 μgm. per ml. only 2 strains grew more than 10 colonies (11 and 12). In the presence of the higher concentrations up to 100 μgm. per ml. the numbers were less variable; only 1 colony grew in 100 μgm. per ml. from each of 2 strains, and a few colonies grew from 15 strains in the presence of 25 μgm. per ml. When the number of colonies growing after 25 days' incubation were compared with those growing at 40 days it became apparent that many of these resistant colonies were dysgonic. The numbers of colonies growing in the presence of concentrations of streptomycin ranging from 1 to 2,000 μgm. per ml. showed that the incidence of resistant colonies was less with this drug and the differences between the numbers growing at 25 days and 40 days were also less, showing that these variants were less dysgonic.

The results are discussed at some length and the need for standard criteria of resistance is emphasized.

S. R. M. Bushby

BELL, W. J. & BROWN, P. P. **Bacterial Resistance in Cases of Pulmonary Tuberculosis in Accra.** *West African Med. J.* 1961, June, v. 10 (n.s.), No. 3, 140–47.

147 specimens of sputum were obtained from patients reporting to Accra tuberculosis clinic and suspected of tuberculosis but denying having had chemotherapy. The specimens were treated with sodium triphosphate, cultured on Löwenstein-Jensen medium and 60 grew tubercle bacilli. Sensitivity tests were then done on the same medium in which the drug had been incorporated in varying concentrations and strains that grew in concentrations 4 times stronger than the minimum inhibiting concentration which inhibited the growth of H37Rv were regarded as resistant. 430 specimens from patients who had received chemotherapy were similarly examined and 127 grew tubercle bacilli.

None of the strains were resistant to cycloserine, ethionamide, TB/1 [thiacetazone] or viomycin and the results with the routine chemotherapeutic drugs were:—

Drug	Resistant strains in:—	
	60 positive cultures from Untreated patients	127 positive cultures from Treated patients
Streptomycin	5 (8%)	32 (25%)
PAS	2 (3%)	22 (17%)
Isoniazid	11 (18%)	66 (52%)

This survey was made during December 1959 to March 1960 and the patients had been treated for varying periods and for upwards of 1 year, the standard treatment had been Isobenzacyl (a combination of isoniazid and PAS in tablet form). It was not possibl to obtain reliable evidence that the untreated excretor of resistant strains had been in contact with know cases of drug resistance, and thus to account for th relatively high incidence of resistant strains in th untreated patients. There was, however, a hig incidence of known contact with patients undergoin chemotherapy but some of the resistance may hav been due to illicit treatment.

Catalase tests made on the isoniazid-resistant culture showed that this enzymatic activity decreased as th resistance increased. *S. R. M. Bushby*

GRIFFITH, L. J., DIETRICH, A. M., Jr., DENARO, S. A. & WILL, F. E. **Altered Guinea Pig Response t Tubercle Bacilli following Prior Infection wit Drug-Resistant Bacilli.** *J. Infect. Dis.* 1961, Jan.–Feb., v. 108, No. 1, 53–8, 6 figs. [19 refs.]

The study described in this paper was designed t see whether the presence of isoniazid-resistant tubercl bacilli affects the course of the disease produced b isoniazid-sensitive, virulent bacilli. 4 groups of 1 guineapigs were infected with 2·6 mgm. of a varian of H37Rv which was resistant to 25 μgm. isoniazi per ml.; this strain did not produce progressive diseas for although tuberculous lesions were found at th site of injection and in the lymph glands, spleen an liver of the group of animals killed after 21 days, ther was little evidence of disease in the animals killed afte 110 days. Tubercle bacilli were, however, sti recovered from the organs of 5 animals and from th injection site of all the 10 animals killed at 110 days At this stage the remaining animals were re-inoculate with 0·9 mgm. of isoniazid-sensitive, fully virulen tubercle bacilli.

The animals of the third group were killed 21 day after the re-infection and they all showed evidence o tuberculosis but it was very much less than in contro animals infected at the same time. 5 of the fourt group were killed 42 days after infection, and althoug they all appeared healthy they had varying degrees o infection; the remaining animals were killed afte another 14 days, and they showed generalized tubercu losis. Cultures were made but no isoniazid-resistan organisms were recovered from the animals killed o the 21st, 42nd and 56th days; the organisms were a sensitive.

The authors conclude that the primary infectio modified the subsequent infection with the virulen bacilli, presumably by stimulating immunity; they were however, unable to explain the disappearance of th isoniazid-resistant bacilli during the infection with th virulent bacilli. *S. R. M. Bushby*

CHRISTENSEN, P. A. **The Drying of BCG Suspension by Evaporation.** *Acta Tuberculosea Scandinavic* 1961, v. 40, No. 2, 109–12, 1 fig.

BCG in concentrations of 20 μgm. per ml. and su pended in various diluents was dried in ampoules fro the liquid state and its viability then compared wit

that of freeze-dried suspensions. The drying was accomplished on a manifold connected to a vapour condenser immersed in alcohol at −23°C. The atmospheric pressure was reduced to a pressure at which degassing commenced, but which was too high for snap-freezing, *i.e.* about 20 mm. Hg. The ampoules appeared to be dry after a few hours and after 24 hours the pressure was lowered to 0·5 mm. Hg. Secondary drying was accomplished over P_2O_5 and the ampoules were then filled with dry nitrogen and sealed.

Viability counts showed that the loss during drying was little reduced if buffer diluents with a *p*H range of 4·5–7·7 were used instead of distilled water, but that the addition of sucrose, glucose or lactose was definitely beneficial, and 6% glucose was chosen for the later experiments. The addition of 3% dextran, 25% nutrient broth or especially 5% gelatin gave slight protection but the results were not followed up. The comparative viability of the same suspension dried by evaporation and by freeze-drying showed that whereas after 4 months at 2°–5°C. the number of viable units of the former was reduced to 30% of the pre-drying value, that for the freeze-dried portion was reduced to 10%. On storing at 20°–30°C. there was not a great difference between the loss of viability of the two preparations but the speed of loss was much greater with the freeze-dried suspension.

The author points out that this estimate of viability is based on the number of viable units present and that the state of aggregation of the bacilli undoubtedly affects the result. However, dispersion was good, especially with the suspension stored at the lower temperature. *S. R. M. Bushby*

UNGAR, J., THOMAS, Valerie & MUGGLETON, P. W. **Freeze-Dried B.C.G. Vaccine from an Isoniazid-Resistant Strain. A Laboratory Investigation.** *Brit. Med. J.* 1961, May 27, 1498–500.

A variant of BCG resistant to 25 μgm. isoniazid per ml. was produced by serial passage in media containing increasing concentrations of isoniazid. It was catalase-negative and dysogonic but its virulence in guineapigs, shown by its ability to produce a local lesion and enlargement of the draining lymph nodes, was unimpaired.

A freeze-dried vaccine was prepared from a culture grown in a modified Sauton medium, and viable counts made over a period showed that the vaccine retained its viability when stored at 4°C. This vaccine when injected in graded amounts into guineapigs, half of which were then treated daily with 20 mgm. isoniazid per kgm., produced tuberculin conversion to 10 TU of PPD, in both groups of animals even when the vaccination dose was 0·1 ml. of 10^{-4} dilution. In contrast, the original isoniazid-sensitive vaccine produced tuberculin conversion, but of a low degree, when the vaccine was diluted to 10^{-1} but not when diluted to 10^{-2}.

Although the stability of the isoniazid-resistance of this variant is as yet unknown, the use of vaccines from isoniazid-resistant variants of BCG should be satisfactory for vaccination of tuberculin-negative contacts while they are on prophylactic doses of isoniazid. *S. R. M. Bushby*

(L. 438)

HUANG, Chien-Tao. **Studies on the Relation between the Age of the Cultures of BCG and *in vitro* Properties of the Vaccine. I. Optical Density and the Total and Viable Number of Bacillary Particles of the Vaccine and Recoverability of the Organisms in relation to the Age of Cultures.** *J. Formosan Med. Ass.* 1960, Oct. 28, v. 59, No. 10, 1124–37, 7 figs. [20 refs.] **II. 2, 3, 5 Triphenyltetrazolium Chloride (TTC) reducing Power and Respiratory Capacity of BCG and its Viability and Recoverability in connection with the Age of the Cultures.** *Ibid.*, 1139–51, 10 figs. [25 refs.]

In the first of these 2 articles the author showed that when BCG was collected as a surface growth from Sauton medium, ball-milled and suspended in phosphate buffer, the optical density increased progressively as the incubation of the culture was extended from 7 to 28 days. This increase was accompanied by an unexpectedly large increase in the total number of bacillary particles per mgm., determined in a counting chamber, but it was not paralleled by a corresponding increase in the number of viable units per mgm.; maximum viability occurred in 7–9-day-old cultures.

In the second study the optical density and viability per mgm. of semi-dry weight were compared with the dehydrogenase activity, measured by the rate of formation of formazan from 2, 3, 5-triphenyl-tetrazolium chloride, and the oxygen-uptake per mgm., determined in the Warburg apparatus. The results showed a close relationship between both of these activities and viability, but with the rate of oxygen-uptake giving the better correlation.

These results are discussed but no satisfactory explanation is offered for the increase in optical density. Cultures aged 7–9 days are recommended for BCG vaccines. *S. R. M. Bushby*

i. VIRTANEN, S. **The Occurrence of Atypical Acid-Fast Organisms in Clinical Samples.** *Acta Tuberculosea Scandinavica.* 1961, v. 40, No. 3, 171–4. [18 refs.]

ii. ——. **Some Characteristics of Atypical Acid-Fast Organisms.** *Ibid.*, 175–81. [40 refs.]

iii. ——. **Drug Sensitivities of Atypical Acid-Fast Organisms.** *Ibid.*, 182–9. [15 refs.]

iv. ——. **On the Importance of Atypical Acid-Fast Organisms as Pathogenic Agents.** *Ibid.*, 190–94.

i. "Acid-fast bacilli were isolated from 1,501 (6·7%) out of 22,380 cultures for tubercle bacillus, 1,411 (94·0%) were typical tubercle bacilli of human type; 90 (6·0%) were atypical acid-fast organisms.

"Of the acid-fast bacilli isolated from gastric lavage 22·3 ± 5·5%, and from the voided urine samples 19·9 ± 2·2%, were atypical acid-fast organisms. They were encountered in only 1·1 ± 1·1% of the catheterized urine samples and in 0·8 ± 0·3% of the sputum specimens."

ii. "A study was made of the growth qualities of 90 atypical acid-fast organisms at 44°C., 37°C., 28°C. and 20°C., their growth on sheep blood-agar plate and in Besredka's liquid culture medium, and their catalase activity.

"With the exception of 2, they even grew at room temperature. 82 strains turned Besredka's culture medium turbid.

"76 strains showed vigorous catalase activity which usually persisted in the culture growing on medium containing 59 mcg of INH. 12 strains, including 11 scotochromogens, showed a weak catalase activity, and the majority of them were completely negative when grown on a medium containing 50 mcg of INH.

"Certain properties which did not concur with the characteristics of atypical acid-fast organisms reported earlier are discussed.

"The most difficult group from the differential diagnosis point of view consists of non-photochromogens. They often failed to show any distinct difference from the 'true' tubercle bacilli in the appearance of their colonies and in their pigmentation."

iii. "The sensitivity of 90 atypical acid-fast organisms to INH, PAS, SM, VM and that of 46 strains to Tb 1 was studied. All the strains with a few exceptions were resistant to INH and Tb 1. Approximately a third of the strains were sensitive to PAS and SM and two-thirds to VM. Sensitivity determinations were performed for 43 strains against chloramphenicol, erythromycin, kanamycin, neomycin, novobiocin, nystatin, oleandomycin, polymyxin B, sulphadiazine and tetracycline. All the strains studied were resistant to nystatin and polymyxin B. There was variation in regard to the other drugs. About three-fourths of the strains were sensitive to sulphadiazine and kanamycin."

iv. "From 88 patients the writer isolated 90 atypical acid-fast organisms of the following types: 3 photochromogens, 38 scotochromogens, 41 non-photochromogens, and 8 rapid growers. Their incidence was the same among both sexes. No correlation to chemotherapy was noted. In no case could any definite etiological significance be attributed to the atypical acid-fast organisms found."

BOJALIL, L. F. **Frequency and Epidemiologic Significance of Unclassified Mycobacteria in Mexico.** *Amer. Rev. Respiratory Dis.* 1961, Apr., v. 83, No. 4, 596–9. [18 refs.]

From 3,315 specimens of sputum or gastric aspirates, 2,437 of which came from patients with proved tuberculosis, 160 cultures of unclassified mycobacteria were grown. 34 of these were scotochromogens, 15 having grown in the presence of tubercle bacilli, 23 were non-photochromogens, 12 having grown in the presence of tubercle bacilli, and 103 were rapid growers, 44 having grown in the presence of tubercle bacilli. Only a few of these were apparently related to pathological lesions; 3 rapid growers were from children with pulmonary lesions, and another was from the cerebrospinal fluid of a child; one scotochromogen was from an abscess of the spinal cord, and 2 others were from children with cervical adenitis, and 3 non-photochromogens appeare to be related to the pulmonary lesions of adult Scotochromogens were relatively common in patien with non-tuberculous pulmonary disease such a chronic bronchitis, who had been treated for prolonge periods with chloramphenicol or tetracyclines.

S. R. M. Bushby

EIDUS, L., MANIAR, A. C., DIENA, B. B. & WALLACE, R **Glycerol Utilization by Mycobacteria.** *Zent. Bakt.* I. Abt. Orig. 1961, v. 182, No. 2, 225–3 2 figs. [20 refs.]

Various strains of mycobacteria were grown as sub merged growth and as surface growth in Sauto medium containing 6% glycerol for 4–8 weeks, afte which the residual glycerol was estimated chemicall and biologically and the quantity of growth wa determined gravimetrically.

The results showed that all the strains examine utilized glycerol to a varying degree. The 8 rapidly growing saprophytes rapidly exhausted the mediu and utilized the highest amount of glycerol per weigh of growth; of these saprophytes, *Mycobacterium phl* was the poorest utilizer. The 14 unclassified strain examined varied in glycerol consumption, with the non chromogenic strains utilizing less than the chromogeni ones. Grown in the pellicle form, the 36 strains o human tubercle bacilli utilized much more glycero than did 11 of the 13 bovine strains; the 2 exceptio being BCG and a strain that had been grown o glycerol medium for some years. When grown i submerged culture there was, however, little differenc between these mammalian strains, except that 2 bovin strains, B143 and B298, behaved like the 11 avia strains in that although they exhausted the glycero supply within 4 weeks, the amount utilized per gm. o growth was relatively low.

S. R. M. Bushby

TODA, T., HAGIHARA, Y. & TAKEYA, K. **A Simpl Urease Test for the Classification of Myco bacteria.** *Amer. Rev. Respiratory Dis.* 1961, Ma v. 83, No. 5, 757–61. [17 refs.]

This note reports the results of the 3 experiment the first of which was designed to show the effects o varying the amount of buffer in the urea substrate an the second to show whether a finely dispersed su pension of the bacilli gave a stronger reaction than coarse suspension. From the results of these 2 exper ments the authors recommended that the substra should consist of 3% urea in a mixture of 0·01 M NaH_2PO_4 and 0·01 M K_2HPO_4 in distilled water, an that the method for testing for urease should consi of adding 0·3 ml. of a bacillary suspension (10–2 mgm. per ml.) and 1 drop of 0·1% phenol red to 3 m of the substrate, and incubating the mixture for 3 da at 37°C. The condition of the suspension is apparentl not important.

In the third experiment, 141 strains of mycobacteri and 1 strain of *Nocardia* were examined by th method; positive results were obtained with human an

bovine tubercle bacilli, photochromogens, some scotochromogens and most saprophytes, but all the avian tubercle bacilli, non-chromogens, some scotochromogens and a few saprophytes gave negative results.

S. R. M. Bushby

Boisvert, H. L'identification des mycobactéries par l'épreuve de réduction des nitrates. **[Identification of Mycobacteria by means of Nitrate Reduction]** *Ann. Inst. Pasteur.* 1961, Mar., v. 100, No. 3, 352–7.

In this method 10–15 mgm. of bacillary growth are suspended in 1 drop of distilled water, and 2 ml. of M/100 $NaNO_3$ in M/45 phosphate buffer *p*H 7 are then added. After the mixture has been incubated at 37°C. for 2 hours 1 drop of concentrated hydrochloric acid, 2 drops of 0·2% sulphanilamide and 2 drops of 0·1% N-(1-naphthyl)-ethylene diamine dihydrochloride are added, and a positive reaction is shown by a purple colour. When the growth is insufficient to yield 10–15 mgm. the test can be done in the culture tube; in the case of fluid culture (Dubos or Youmans media) the quantities of reagents per 5 ml. of media are 2 drops of 4·25% sodium nitrate, 3 drops of the acid and 5 drops of the coupling reagent.

These methods are based on those of Virtanen [this *Bulletin*, 1960, v. 36, 1136], and the results on a variety of mycobacteria confirm his findings. 131 strains of human tubercle bacilli, 70 of which were isoniazid-resistant, 29 of 30 strains of photochromogens, and most of 30 rapid-growing mycobacteria and 5 pathogenic strains of *Nocardia* gave positive results, whereas 48 of 50 strains of bovine tubercle bacilli, 1 of BCG, 5 of vole bacilli, 2 of *Mycobacterium johnei*, 1 of *Myco. ulcerans*, 2 of *Myco. balnei*, 1 of *Myco. chelonei* and 1 of *Nocardia rhodocrans* gave negative results. Of 16 strains of avian tubercle bacilli 13 were negative. Of 40 strains of scotochromogens, 30 were negative, 8 were positive and 2 were doubtful. Of 26 non-chromogens, 12 were positive, 11 were negative and 3 were doubtful.

The author maintains that the test should always be done along with the niacin test.

S. R. M. Bushby

Artman, M. & Bekierkunst, A. ***Mycobacterium tuberculosis* H37Rv Grown *in vivo*: Nature of the Inhibitor of Lactic Dehydrogenase of *Mycobacterium phlei*.** *Proc. Soc. Exper. Biol. & Med.* 1961, Mar., v. 106, No. 3, 610–14, 1 fig.

Previous reports have shown that H37Rv grown *in vivo* (LH37Rv) absorb from the lung tissue of tuberculous mice an inhibitory substance which inhibits the electron transport system of cell-free extracts of mycobacteria, and that extracts from various organs of normal mice, and especially tuberculous mice, possess a similar inhibitory property. The purpose of the present study was to show whether the inhibition was due to identical factors. The effects on the electron transport system were measured by the amount of formazan formed from triphenyl-tetrazolium chloride by lactic dehydrogenase in the presence of sodium lactate.

The first experiments using the electron transport system of *Mycobacterium phlei*, showed that the inhibitor from the organs of mice and from the surface of LH37Rv was destroyed by heating to 80°C. for 3–5 minutes and that dialysis did not affect the activity. Pre-incubation of cell-free extracts of *Myco. phlei* with sub-effective concentrations of the extracts showed a gradual decrease in dehydrogenase activity of the mycobacterium at 37°C. but not at 4°C., suggesting destruction of a component of the dehydrogenase system. The dehydrogenase activity was restored by the addition of boiled extracts of *Myco. phlei*, but not by dialysed extracts; restoration of activity was, however, not attained if the boiled extracts were incubated with the inactivating extracts. Adding the known coenzymes of lactic dehydrogenase to extracts of *Myco. phlei* which had been inactivated by LH37Rv cells showed that the addition of diphosphopyridine nucleotide (DPN) completely restored activity, and that pre-incubation of the DPN with LH37Rv cells, spleen or lung extracts destroyed the restorative property.

Since DPN is known to be destroyed by either the nucleotide pyrophosphatase or pyridine transglycosidase, experiments were performed with both enzymes to determine which is operative in these experiments. The results showed clearly that DPN was hydrolysed at the nicotinamide ribose linkage, and therefore that inhibition by LH37Rv, lung and spleen extracts is due to the activity of DPNase present in all these preparations.

The authors suggest that the inhibitor adsorbed on to the tubercle bacillus *in vivo* inhibits, after ingestion, the respiration of the phagocyte.

S. R. M. Bushby

Merkal, R. S. **Separation and Serologic Identification of Fractions from the Culture Filtrate of *Mycobacterium paratuberculosis*.** *Amer. Rev. Respiratory Dis.* 1961, July, v. 84, No. 1, 52–9. [14 refs.]

Nagayama, H., Konno, K. & Oka, S. **Formamidase in Mycobacteria and its Use in differentiating Saprophytic Mycobacteria from other Mycobacteria.** [Correspondence.] *Nature.* 1961, June 24, v. 190, 1219–20. [14 refs.]

4 human strains, 4 bovine strains and 2 avian strains of tubercle bacilli, 2 photochromogens, 1 scotochromogen, 2 non-photochromogens, 1 strain of *Mycobacterium fortuitum*, and 7 strains of saprophytic mycobacteria were tested for formamidase by taking the surface growth from Sauton medium, grinding the bacillary mass in sand, extracting it with water and then incubating the extract in a phosphate buffer at *p*H 7·0 with formamide. After 4 hours' incubation the mixture was examined colorimetrically for ammonia. Only the saprophytes gave a positive result.

An examination of the enzyme showed that 79·3% of the substrate was split within 2 hours, the rate of

splitting remaining constant over this period; the rate was maximum in the presence of 5 μmoles formamide per ml. Dialysis against distilled water did not affect the activity. The enzyme was also active towards nicotinamide, pyrazinamide, benzamide, *iso*-nicotinamide, L-asparagine and urea. It was active over the *p*H range of 6·0–9·0, this *p*H activity range being similar to that of nicotinamidase but different from those of benzamidase and urease. Heat stability studies suggested that the enzyme is not identical with any of the other known amidases.

S. R. M. Bushby

WAGNER, J. C. & BOKKENHEUSER, V. **The *Mycobacterium* isolated from the Dassie *Procavia capensis* (Pallas).** *Tubercle.* 1961, Mar., v. 42, No. 1, 47–56, 5 figs. [17 refs.]

This is a further report on the character of the mycobacterium isolated from the Cape hyrax (dassie) [*Procavia capensis*] (See *Nature*, 1958, v. 181, 284). This bacillus was first grown from the local abscess of a dassie inoculated with the granuloma from the primary lung lesion, the bacillus having failed to grow the primary lesion. The initial growth on Dorset egg medium took 16 weeks to become visible, but subcultures grew in 3–4 weeks, and growth was better at 37°C. than at 26°C. Two types of matt colonies were produced, one almost white and raised 0·5–0·8 mm., and the other an irregular flat and off-white colony, 1·2–1·5 mm. Good growth occurred on Löwenstein-Jensen medium, potato agar, Youmans, Petragnani, Kirchner and Dubos media; the addition of glycerol had no effect, and growth was sparse on nutrient agar. The bacillus was neutral red positive but it did not cord. Its growth was inhibited by 25 μgm. streptomycin per ml., 1 μgm. isoniazid per ml. and 0·4 μgm. PAS per ml.

In guineapigs, rabbits, mice, *Mystromys albicaudatus*, *Rattus natalensis* and *Tatera brantsi*, it usually caused only a local lesion, and no lesions were produced in fowls. In mice, vaccination with the bacillus afforded some protection against the vole bacillus.

Although the bacillus could be one that did not cause the original granulomatous lesions, the authors think this possibility unlikely; they consider the bacillus as either an attenuated strain of the vole bacillus or as an independent species.

S. R. M. Bushby

CALALB, G., SARAGEA, Alice & MAXIMESCO, Paula, with the collaboration of Natalia CIOROIANU, Ana POPESCO, Suzana POPA & Ana MIHĂILESCO. Recherches sur un milieu liquide d'enrichissement pour le diagnostic bactériologique de la diphtérie. **[Investigation of a Liquid Enrichment Medium in the Bacterial Diagnosis of Diphtheria]** *Arch. Roumaines Path. Expér. et Microbiol.* 1961, Mar., v. 20, No. 1, 95–101. [18 refs.]

It is recommended that, especially for the detection of carriers, a fluid medium of the following composition is valuable:—

Ox serum	150 ml.
2% Glucose broth	50 ml.
Egg yolks	2
2% sol. of potassium tellurite	20 ml.
10% cystine sol.	2·2 ml

A preliminary incubation in this medium is made an the fluid cultures are inoculated to solid media, prefe ably the Gundel-Tietz blood tellurite agar. Results a recorded for the examination of 195 contacts and 3 cases of diphtheria. Successful isolations were invar ably higher if plating on a solid medium followed preliminary incubation in this fluid than on dire plating to the same solid medium. The superiority the results varied from 10% to 25%.

J. W. McLeod

SEVER, J. L. **Diphosphopyridine Nucleotide Bindin Effect of Sickle Cell Erythrocytes on *in vitr* Growth of *Hemophilus influenzae*.** *Proc. So Exper. Biol. & Med.* 1961, May, v. 107, No. 23–6.

As an auxiliary growth factor for the growth of *H influenzae*, the blood (AS) of sickle-cell trait carrie was normal, while the blood (SS) of persons with sickl cell anaemia was defective. This defect was comple if the cells had stood for 1 hour before incorporatio into the medium, but only partial if the cells were use at once. Normal (AA) and AS cells lost their activit only after standing for 6 hours. Haemolysis of any these types of cell, by any of the usual method abolished their growth-supporting powers. But if fres cells (including SS cells) were "chocolated" imm diately, they fully supported growth. A red cell su pension or a haemolysate whose activity had decline to zero could be restored to full activity by pept digestion—and this applied to SS as well as to AS an AA cells. In the case of haemolysates, it was th stromal residue, and not the supernatant, that could b re-activated by digestion. The addition of diphosph pyridine nucleotide (DPN) also restored full activity inactive preparations; but it required 8 times as muc DPN to restore SS cells as it did to restore AA and A cells.

These results are interpreted as evidence of the bin ing of DPN by red cell stroma, a process much mo active for SS than for other cells; but the reason f this remains obscure. [It would be of interest to kno whether other anaemias accompanied by a high reticul cytosis resembled sickle-cell anaemia in this respect

Alan B. Raper

GOODGAL, S. H. & HERRIOTT, R. M. **Studies on Tran formations of *Hemophilus influenzae*. I. Com petence.** *J. General Physiol.* 1961, July, v. 4 No. 6, 1201–27, 6 figs. [13 refs.] **II. The Mol cular Weight of transforming DNA by Sed mentation and Diffusion Measurements.** *Ibi* 1229–39, 2 figs. [17 refs.]

ORFILA, Jeanne & COURDEN, B. Contribution au diagnostic différentiel d'*Hemophilus influenzae* et d'*Hemophilus aegyptius*. [**Differential Diagnosis between *Haemophilus influenzae* and *Haemophilus aegyptius***] *Ann. Inst. Pasteur.* 1961, Feb., v. 100, No. 2, 252–6, 1 fig.

The authors have shown another method of differentiation between *Haemophilus influenzae* and *Haemophilus aegyptius*. Mice (25 gm. male) were injected intravenously with 0·025 ml. of a 1/10 dilution of a saline suspension of each organism. The suspension was made from cultures 24 hours old grown on brain heart infusion agar + cell extract suspended in saline and calibrated to 70 for the wavelength of 660 in the Bausch and Lomb spectrophotometer. 3 minutes after injection and at regular intervals, blood was taken from the mice and diluted in 2·5 ml. of broth for the first sample and in 0·25 ml. of broth for subsequent samples. 0·25 ml. of the diluted samples were spread over the surface of Petri dishes containing the solid medium. After 48 hours' incubation colony counts were done and the results plotted against time.

H. aegyptius disappeared rapidly from the blood after 6 minutes. At 20 minutes the blood was sterile and remained so, the mice recovered rapidly and there were no deaths.

H. influenzae also decreased rapidly in the first 6 minutes but did not entirely disappear; at 20–40 minutes the numbers were stationary, and then increased till they were equal or greater than the initial amount. The mice died in 4 days with *H. influenzae* septicaemia. The viscera served as a focus of bacterial multiplication.

It is essential that the mice should be free from latent *Haemophilus* infection as healthy mice which were carrying a dormant *H. influenzae-murium* when injected with *H. aegyptius*—both live and heat-killed cells—gave a result similar to that produced by *H. influenzae* infection. [The curves of the graph in Fig. I are wrongly labelled.] *Elizabeth M. Harper*

LIU, P. V., ABE, Y. & BATES, Janice L. **The Roles of Various Fractions of *Pseudomonas aeruginosa* in its Pathogenesis.** *J. Infect. Dis.* 1961, Mar.–Apr., v. 108, No. 2, 218–28, 3 figs. [13 refs.]

This is a study of the pathogenicity of *Pseudomonas aeruginosa* [perhaps more generally known as *P. pyocyanea*], by examination of the properties of various fractions extracted from cultures of the organism grown on Cellophane plates overlying a nutrient agar medium described in 1957 by LIU [*J. Bacteriology*, 1957, v. 74, 718; see also this *Bulletin*, 1958, v. 33, 797]. The technique for the preparation of 6 fractions is described in the present article: fraction Ia = pyocyanin; Ib = all dialysable substances other than pyocyanin; II = haemolysin; III = extracellular enzymes; IV = slime; V = bacterial cells.

Slime (fraction IV) appeared to be the most important fraction isolated. It was toxic for mice and antigenic. The slime antigen was, however, type-specific in as much as all strains of *P. pyocyanea* did not produce slime with the same antigenic composition. The antiserum prepared from this slime antigen agglutinated its own bacterial cells and protected mice against infection by the homologous strain of *P. pyocyanea* or infection with the same antigenic type of slime. Both the haemolysin and the extracellular enzymes were toxic for animals but only the latter were antigenic and they, too, were species-specific. The bacterial cells were relatively non-toxic and the endotoxin did not appear to have the significance that belongs to the endotoxin of the typhoid bacillus. Fraction Ib was found to cause necrosis of plant tissue but it was not toxic for animals. Fraction Ia, pyocyanin, was toxic to tissue cultures of mammalian cells grown *in vitro*, but when injected into animals there was little or no evidence of toxic effects. *H. J. Bensted*

LYSENKO, O. ***Pseudomonas*—an Attempt at a General Classification.** *J. General Microbiol.* 1961, July, v. 25, No. 3, 379–408, 1 text fig. & 4 figs. on pl. [Numerous refs.]

126 strains, representing 46 named and 20 unidentified species of *Pseudomonas* and 6 species of *Aeromonas*, were put through a series of tests useful for identification. Among these tests a new method was developed for detecting the oxidative use of carbohydrates: the organism was grown for 5 days in a glucose-containing medium with and without iodoacetate; a positive result [indicating oxidation] showed acid production in both media, a negative [blockage of glycolytic pathway or fermentation] showed acid only in the tube without iodoacetate. The similarity (and difference) between strains was estimated by using the results of the various characterising tests to calculate the S (similarity) values by SNEATH's [this *Bulletin*, 1957, v. 32, 1181] method. Strains sharing many characters in common had high S values, strains with many differences, low values.

The glucose-fermenting strains were either *Aeromonas* species or were not discussed because they were not pseudomonads. The characters of the glucose-oxidizing strains (pseudomonads) were recorded in long and detailed tables which should be consulted in the original. The species recognized included *P. aeruginosa* (*B. pyocyaneus*), *P. fluorescens* and *P. pseudomallei* (Whitmore's bacillus). Type strains were chosen for 17 of the species [but adequate reasons are not given for rejecting the neotype for *P. fluorescens* chosen by RHODES]. *S. T. Cowan*

ANN. INST. PASTEUR. 1961, June, v. 100, No. 6, Suppl., 122 pp., illustrated. [Numerous refs.] Colloque annuel de la Société Française de Microbiologie, 10 janvier 1961 [FASQUELLE, R., President]. Les problèmes actuels de l'identification des *Pseudomonas*, des *Achromobacter*, et de bactéries analogues. [**Problems in the Identification of *Pseudomonas*, *Achromobacter* and Analogous Bacteria**].

[The French Microbiological Society chose a difficult subject for their 1961 meeting; difficult because the different speakers did not mean the same things when

they used the same bacterial names and gave different names to similar organisms. In reading the following abstract the reader should bear in mind that *Achromobacter* (Buttiaux) = *Acinetobacter* (Brisou) = *Moraxella* (Piéchaud) = *Alcaligenes* (Thibault). A fine disregard for the rules of nomenclature was accompanied by "la conception taxinomique française" to what should be an approach above nationalities.]

R. FASQUELLE (*Prologue*, pp. 7–9) and L. ENJALBERT (*Problèmes actuels de l'identification des Pseudomonas, des Achromobacter et des bactéries analogues*, pp. 10–15) think that *Pseudomonas, Achromobacter* and allied organisms have some medical significance and list the type of disease they may produce [pseudo-typhoid by *Achromobacter alcaligenes* is one example].

M. VÉRON (*Pseudomonas pigmentés*, pp. 16–42) excludes the production of soluble pigment from the essential characters of the genus *Pseudomonas*. Motility, however, is essential and is always due to polar flagella; *Ps. aeruginosa* has one polar flagellum, *Ps. fluorescens* one or more. Pseudomonads are strict aerobes, catalase-positive, oxidase-positive, citrate-positive, lysine decarboxylase-negative, and they oxidize carbohydrates. Variable characters, which may be of value in distinguishing species, are haemolysis, lecithinase, peptonization of milk, hydrolysis of gelatin and urea, H_2S production, nitrate reduction and pathogenicity to guineapig or rabbit.

Most strains of *Ps. aeruginosa* are lysogenic and the phages are inactive against *Ps. fluorescens*. 90% of strains fall into 12 serological O groups. Véron does not think it is possible to define a group of phytopathogens and recognizes only two species, distinguished by growth (or absence of growth) at 42°C., lecithinase, sensitivity to phages, and somatic antigens.

R. BUTTIAUX (*Pseudomonas non pigmentés et Achromobacter*, pp. 43–58) does not exclude non-pigmented strains from *Pseudomonas* if they have the other characters of the genus. One point he makes is that pseudomonads usually attack lactose slowly. *Achromobacter* he defines as coccobacilli, non-motile, usually Gram-negative but may occasionally retain some purple stain, non-pigmented, strict aerobes. They do not attack glucose, and make HUGH and LEIFSON medium [this *Bulletin*, 1954, v. 29, 83] alkaline, and may be oxidase-positive or negative. These characters do not correspond with *Achromobacter* in Bergey's *Manual*. Non-motile strains of *Alcaligenes* are included but motile strains of *Alcaligenes faecalis* are regarded as non-pigmented pseudomonads or vibrios.

P. THIBAULT, in *A propos d'Alcaligenes faecalis* (pp. 59–73) says that after 60 years of discussion even its morphology is not settled. Of 53 strains examined only 13 had peritrichous flagella and thus conformed to the original description. [The legends of Tables I and II are inaccurate and do not agree with the text.] 6 strains oxidized maltose and corresponded to *Ps. maltophilia* Hugh and Ryschenkow.

M. PIÉCHAUD (*Le groupe Moraxella. A propos des B5W-Bacterium anitratum*, pp. 74–85) divides *Moraxella* into two groups [only one of which would generally be accepted as *Moraxella*]: I, oxidase-positive, nitrate-positive, penicillin-sensitive; II, oxidase-negative, nitrate-negative, penicillin-resistant, and in group I places the glucose-oxidizing, oxidase-negative *Bact. anitratum* under the name *M. glucidolytica* [see also LAUTROP, *Internat. Bull. Bact. Nomencl. Taxon.*, 196 v. 11, 107].

J. BRISOU (*Étude antigénique des Achromobactereae* pp. 86–99) has a different conception of *Achromobacte* as a genus in a tribe Achromobactereae in the famil Pseudomonadaceae. It it are motile or non-motile non-chromogenic Gram-negative rods that one fee should be Enterobacteriaceae but are not. He include *Erwinia* in the tribe and finds many cross-reactions i agglutination tests between *Achromobacter, Erwinia* an various Enterobacteriaceae.

Sensitivity to antibiotics was reported by Y. CHABBER and A.-L. COURTIEU (*La sensibilité aux antibiotiques de Pseudomonas, Achromobacter et "B5W-Bacterium anitratum"*, pp. 100–113); some strains produced penicillin ase and penicillin sensitivity could not be used as taxonomic character. In the Discussion (pp. 114–122 A.-L. COURTIEU, S. CHASSIGNOL and C. LONGERA (*Caractères bactériologiques de 214 souches de Moraxella lwoffi* [*sic*] *et de M. glucidolytica* (*Acinetobacter*) pp. 116–122) presented another classification in which they recognize a tribe, Moraxelleae, with two genera *Moraxella* (oxidase +) and *Mima* or *Herellea* (oxidase −); in the second group (B5W) the priority of the specific epithet *vaginicola* (over *glucidolytica*) i recognized.

[The meeting showed the wide diversity of view held by workers in this difficult, but medically rather unimportant, group of bacteria.] *S. T. Cowan*

LÁNYI, B. & ÁDÁM, Maria M. **Agar Diffusion Test and Micromethods for the Rapid Biochemical Differentiation of Enteric Bacteria.** *Acta Microbiologica.* Budapest. 1960, v. 7, No. 3, 313–28, 1 fig [70 refs.]

The agar diffusion test in the title of this paper refers to a method of testing the biochemical reactions of Gram-negative intestinal bacteria by the application of small squares of dried filter-paper, which had previously been impregnated with defined concentrations of the fermentable sugars or other reagents, to the surface of a deoxycholate-phenol agar plate freshly inoculated with a pure culture of the organism under test, and reading the results after incubation. The isolated colony on the original plate culture to be identified is first inoculated into a combined urease-indole medium. After a few hours' incubation some of the growth is spread evenly on the surface of the deoxycholate-phenol agar and the glucose paper square is placed adjacent to this inoculated area with the other sugar papers and the H_2S reasonably close. Thus the same plate serves for the demonstration of fermentation, gas and hydrogen sulphide production and also for the determination of the methyl red reaction; the indole and urease reactions are tested separately. The authors report that their method, which is a modification of one originally described by KNOX [this *Bulletin*, 1950, v. 25, 623], gives results which agree very closely with those obtained by the orthodox procedures. *H. J. Bensted*

NICOLLE, P., DIVERNEAU, G., BRAULT, J. & DU PLESSIS, A. M. Sur quelques lysotypes exotiques de *Salmonella typhi* découverts par le centre français de la lysotypie entérique. [**Some New *Salmonella typhi* Phage Types Identified by the French Centre for Enteric Phage-Typing**] *Bull. Soc. Path. Exot.* 1960, Nov.–Dec., v. 53, No. 6, 965–70.

The French Centre for Enteric phage-typing was established in 1948 and since that date has examined some 13,000 cultures of typhoid bacilli sent to it from various parts of the world, a great number coming from the Far East. Many untypable strains were received from Vietnam, Cambodia, Central Africa and Spain and among these, 6 new Vi phage types have been identified—types E-10, J-3, M-2, M-3, 37 and 46. There was also the Central African variety of Vi phage type C-1 which was associated with the serious outbreak of typhoid in Léopoldville in 1958 [this *Bulletin*, 1956, v. 31, 741]. *H. J. Bensted*

NICOLLE, P. & DIVERNEAU, Gisele, with the technical assistance of Janine MARTIN & Anne M. DU PLESSIS. Sur une lysotypie complémentaire des souches Vi positives de *Salmonella typhi*, non lysotypables par la méthode de Craigie et Felix. [**A Complementary Lysotype of Vi-positive Strains of *Salm. typhi*, not Typable by the Method of Craigie and Felix**] *Zent. f. Bakt.* I Abt. Orig. 1961, v. 181, No. 3, 385–7.

CHOMICZEWSKI, J. Badania nad typowaniem bakteriofagowym i biochemicznym *Salmonella typhi*. I. Zmienność obrazu typów bakteriofagowych w środowisku endemicznym. [**Investigations of the Phage- and Biochemical-Typing of *Salmonella typhi*. I. Variability of the Picture of the Phage Types in the Endemic Environment**] *Med. Dośw. i Mikrob.* Warsaw. 1961, v. 13, No. 3, 217–28. [110 refs.] II. Indukowana zmienność typów bakteriofagowych. [**II. Induced Variability of the Phage Types**] *Ibid.*, 229–39, 1 fig. [45 refs.]

The English summaries appended to the papers are as follows:—

I. "The observations of the picture of the phage-types in an endemic environment during five years (1956–1960) showed, that the majority of the types demonstrate a stabilized picture, characteristic for the environment. However some types may show even considerable fluctuations in the spreading and frequency of causing new cases of disease. The epidemiological character of a type (i.e. the frequency of its occurrence in the focuses of the diseased or carriers) is not a constant feature and in the course of a few years may vary essentially. These fluctuations may depend on accidental contacts between the focuses. In the light of the investigations concerning the role of the latent bacteriophages in the antigenic variability in Salmonella it is not possible to exclude for a certainty the existence of immunological differences between the types, causing in certain periods a different state of the environment's immunity against definite types.

"For the differentiation of focuses caused by often occurring types, the new Vi-phage 'Lódz-13' is useful."

II. "The strains of various types of S. typhi in the form of single-cell cultures were irradiated with strong doses of UV rays and exposed to the action of the five principal antibiotics. Under the influence of UV the majority of the types showed unchanged lytic reactions to preparations of the phage Vi II. Only the strains of the types T and 40 changed to a degraded form, moreover their lytic spectrum proved to be identical, corresponding to the spectrum of the degraded strains occurring in natural conditions belonging to the II biochemical type.

"Antibiotics, administered even in very large doses, mostly did not change the typical specificity of the strains. Under the influence of streptomycin a transition of the type 26 into a form very near to type 25 was obtained. The type-determining phage isolated from the changed strain was able to transform the type A into type 26. Therefore the obtained form shows different phenotypical features (corresponding to the type 25) and genotypical ones (characteristic for the type 26), proving thereby, that there exists in the bacterial cell another factor, conditioning the pattern of resistance, independent of the type-determining phage."

BILLAUDELLE, H. & TIRUNARAYANAN, M. O. **Studies on *Salmonella typhi*: 3. Further Fractionation and Biologic Properties.** *Ztschr. f. Immunitätsf. u. Exper. Therap.* 1960, Nov., v. 120, No. 4, 229–37, 3 figs.

The senior author has previously studied the water-soluble extract obtained after repeated freezing and thawing of *Salmonella typhi* [this *Bulletin*, 1960, v. 35, 923; 1961, v. 36, 189] and found that it was immunogenic and elicited antibodies, but that it also contained pyrogenic substances. In the present paper he describes the fractionation of water-soluble extracts from Vi-positive and Vi-negative strains of *Salm. typhi* by means of ion-exchange resins for the preliminary separation into different groups, followed by further purification. The various fractions were then examined for immunogens, agglutinogens and pyrogens. One fraction contained all three, one immunogen and pyrogen, one pyrogen only, and yet another was without any of the three activities. It was not possible to demonstrate immunogenic activity without pyrogenic activity, but agglutinogenic response did not always accompany immunogenic activity. *H. J. Bensted*

GAINES, S. & TULLY, J. G. **Effect of Typhoid O Antigen (Endotoxin) on the Virulence of an O-Deficient Variant of *Salmonella typhosa*.** *Amer. J. Hyg.* 1961, Mar., v. 73, No. 2, 224–30. [21 refs.]

Using *Salmonella typhi*, strain Vi 1 (Bhatnagar), and the cerebral route for inoculation into mice, the authors found that the LD50 for the Vi 1 strain, inoculated

alone was $2{\cdot}93 \times 10^6$, but when this strain was inoculated intracerebrally together with 15 μgm. of purified endotoxin prepared from *Salm. typhi* O-901 the LD50 was reduced to $3{\cdot}32 \times 10^4$ organisms. The Vi 1 strain although very rich in Vi-antigen is almost completely deficient in H and O antigens. Even when the experiment was repeated with typhoid cultures such as strain Ty2 (OVi) and the W variant (which probably contains traces of Vi antigen) the same sort of result was obtained although the difference was not quite so great. The addition of 15 μgm. endotoxin to strain Vi 1 enhanced the virulence of the O-deficient strain more than 80 times, whereas the addition of the same amount of endotoxin to the fully smooth strain Ty2 resulted in a more than 7-fold increase in virulence. It would therefore appear that although it is necessary to inject the isolated O antigen simultaneously with the bacterial cell suspension to enhance its virulence, it need not form an integral part of the bacterial cell itself.

These experiments are of considerable interest and offer convincing proof of FELIX's contentions [this *Bulletin*, 1934, v. 9, 851] about the importance of the O antigen in the virulence of the typhoid bacillus.

H. J. Bensted

GERICHTER, C. B. [**Bacteraemia in White Mice infected perorally with *Salmonella typhosa***] *Harefuah.* Jerusalem. 1961, Aug. 1, v. 61, No. 3, [in Hebrew 89–90. English summary 91].

The English summary appended to the paper is as follows:—

"By administering increasing doses of *S. typhosa* (strain Ty_2) orally to mice it was possible to determine the minimal infective dose for the blood stream. This dose was approximately 50 thousand bacteria.

"20 seconds after the oral administration of a dose of 5×10^9 *S. typhosa* bacteria, these microorganisms had already appeared in the blood stream.

"Between 20–60 seconds 64% of blood samples were found to be positive. After 2–3 minutes 64·5% of blood samples and between 3 minutes and 3 hours 76% of blood samples were positive. After this time there was a gradual decrease in the percentage of positive blood samples and after several days, the blood was found to be sterile."

OLITZKI, A. L., SULITZEANU, D., SHARON, N. & GELERNTER, I. [**The Accumulation of Toxic Substances in the Organs of Mice infected with *Salmonella typhosa* and their Relationship to the Failure of Chloramphenicol Treatment in the Final Stages of the Infection**] *Harefuah.* Jerusalem. 1961, Aug. 1, v. 61, No. 3, [in Hebrew 75–9. English summary 80].

The English summary appended to the paper is as follows:—

"When 2×10^8 or 4×10^8 microorganisms of *Salmonella typhosa* (strain Ty_2) were injected intraperitoneally into white mice, their multiplication was limited and 15 hours after the onset of the infection their tot number in all organs of the host was about 10^{10}. Whe 5×10^3 were injected in a 5% mucin solution the started to multiply immediately and 5 hours lat reached a total count of about 10^6. The peak wa reached after 20 hours when the first deaths occurre In both types of infection soluble bacterial antige appeared in the peritoneal fluid and in the abdomin organs. These antigens gave positive precipitation rea tions with anti-Vi and anti-O immune sera, whic became stronger concurrently with the progress of th infection.

"The presence of these antigens indicated that th living organisms do not contribute the only source o toxic bacterial substances to which the host is expose The living count constitutes merely the result of th steady multiplication and disintegration of infectin organisms *in vivo*, which result in a progressive accumu lation of soluble bacterial substances in the organs o the host. From the primary focus, the abdomin cavity, the micro-organisms migrated and invaded th blood stream and all body organs. This general inva sion into all organs and the multiplication in ther might be made possible by the accumulation of a aggressin-like substance in them. It is not yet clea whether the O- and Vi-antigens alone act as aggressi or whether their action is supported by a specific ant gen which is produced *in vivo*. However, there is n evidence that another toxin is present in addition t the endotoxin.

"Normal mice which had received 0·5 ml. of extrac of infected organs intra-abdominally did not succumb but developed a solid immunity against virulent *typhosa* indicating that the organ extracts contained soluble immunizing substance. The treatment wi chloramphenicol was effective when it was started hours after the onset of the infection with the muc method. At that time the total bacterial count *in vi* was about 10^7. When the mice were treated with sma amounts of chloramphenicol, which just inhibited exce sive multiplication of bacteria, but still maintained level of 10^6–10^7 bacteria for 24–48 hours, the gre majority of the animals survived. On the other han chloramphenicol treatment, started when the *in vi* count had approached 10^9, was useless even if the do given was 10·5 mgm. These findings show that dea was caused by accumulation of a toxic bacteri product.

"In the present state of the investigation we cann yet decide whether this toxin is identical with t endotoxin or whether there exists an additional tox produced only *in vivo*."

RASKA, K., MATĚJOVSKÁ, D. & JELÍNEK, J. **The Labor tory Assay of the Potency of Typhoid Vaccin** *Ztschr. f.d.g. Hyg. u. ihre Grenzgebiete.* Berli 1961, Jan., v. 7, No. 1, 34–40, 5 figs.

The authors consider that the interpretation of t mouse protection test for the assessment of potency typhoid vaccines would be much assisted if at appr priate periods after the challenge by intraperitone injection the blood and peritoneal cavity of each mou

ere examined for the presence of the challenging rganism. The results obtained depend on the degree f immunity induced by the vaccine and the challenge ose; they fell into 4 groups: (a) No reaction, mice urvived and there was no evidence of typhoid bacilli n the blood 24 hours after challenge or in the peritoneum –5 days after challenge. (b) The mice survived and yphoid bacilli were not found in the blood although ney were present in the peritoneum. (c) The mice urvived but typhoid bacilli remained in both the blood nd the peritoneal cavity for the periods stated. (d) he mice died within 4 days with organisms in the lood and peritoneum. The degree of immunity induced y the vaccine depends on the dose injected and prac- ically all mice that had been vaccinated and survived he challenge failed to show any organisms in their lood after an interval of 24 hours.

The method has not yet been applied to comparative rials with different vaccines and the observations refer o the alcoholized typhoid vaccine with the Ty2 strain f *Salmonella typhi*. *H. J. Bensted*

Vulchanov, V. H. & Popivanov, R. **Immune Leukopenia in Rabbits induced with Typhoid Vi-Vaccine.** *Ztschr. f. Immunitätsf. u. Exper. Therap.* 1960, Nov., v. 120, No. 4, 295–309, 4 graphs. [17 refs.]

The effect of alcoholized typhoid vaccine (*Salmonella yphi* strain Ty2) on the leucocytes of rabbits was studied by doing serial differential counts on the blood of abbits at set intervals after the first, second and third njection of the vaccine, in addition to total white cell ounts. The sera of the immunized rabbits were shown o contain complement-fixing antibodies against leuco- ytes and the titre of the sera was related to the degree f leucopenia (immune leucopenia). This antityphoid Vi) serum not only has the power of binding extracts f rabbit leucocytes in the complement-fixation test, ut also human leucocytes, although the titres of the nti-vaccine sera in the CF tests were lower than those ound in the sera of rabbits immunized with human eucocytes. The sera of rabbits immunized with typhoid- Vi vaccine also reacted with extracts of human, rabbit nd guineapig erythrocytes but the titres of these ietero- and iso-erythro-antibodies were significantly ower. *H. J. Bensted*

Bonnefoi, A., Le Minor, L. & Le Minor, S. Courbes d'agglutinines obtenues chez les lapins immunisés au moyen de différents vaccins antityphoidiques. **[Agglutinin Curves obtained in Rabbits immunized with Different Typhoid Vaccines]** *Ann. Inst. Pasteur.* 1961, Apr., v. 100, No. 4, 406–19, 9 figs.

All the typhoid vaccines used in the experiments described in this paper employed the Ty2 strain of yphoid bacillus but the methods of preparation were different; they included treatment with alcohol, phenol, eat, toluene, acetone, and calcium chloride. It will be ecalled that Ando and Nakamura [this *Bulletin*, 1952, v. 27, 913] showed that $CaCl_2$ was a valuable stabilizing agent for Vi antigen for agglutination tests. If $CaCl_2$ is added to fresh living suspensions of the Ty2 strain it will withstand heating without loss of Vi antigen.

Groups of rabbits were immunized with the various vaccines and blood was taken after 7 days, 1 month, 3 months, 6 months, 9 months and after a booster dose, and examined for H, O and Vi agglutinins. The outstanding feature noted was the high level of Vi agglutinins in the rabbits injected with the $CaCl_2$-treated vaccine. Not only was the immediate response very good but the levels were still high 9 months after primary vaccination. The booster dose produced a steep rise in Vi agglutinins although they did not reach the high levels obtained 7 days after the primary course of injections. *H. J. Bensted*

Kato, T. & Funaki, I. **Bactericidal Action of the Intestinal Tissue of Rabbits immunized by *Salmonella typhi*.** *Wakayama Med. Reps.* 1960, Oct., v. 5, No. 2, 201–9, 1 fig.

The authors found that the intestinal mucosa of normal healthy rabbits would yield an extract which promoted the growth of typhoid bacilli, but that extracts prepared from the intestinal mucosa of rabbits immunized against typhoid bacilli, the latter part of the immunization scheme including oral administration of suspensions of living organisms, contained some substance which was bactericidal for *Salmonella typhi*. Specific antibody could not be demonstrated in the extract from the immune rabbits' mucosa but this extract produced marked morphological changes in bacterial suspensions of *Salm. typhi*; the bacteria were generally shorter and fatter; coccobacillary forms were common. The staining properties were irregular, there was evidence of vacuolation and other signs of degeneration. It would appear that these results are put forward as examples of local immunity in the small gut of rabbits as a result of anti-typhoid immunization by the oral route. *H. J. Bensted*

Falkow, S., Marmur, J., Carey, W. F., Spilman, W. M. & Baron, L. S. **Episomic Transfer between *Salmonella typhosa* and *Serratia marcescens*.** *Genetics.* 1961, July, v. 46, No. 7, 703–6. [12 refs.]

Lehnert, C. & Bathke, W. Schildkröten als latente Salmonellenausscheider. **[Tortoises as Latent Carriers of Salmonellae]** *Ztschr. f.d.g. Hyg. u. ihre Grenzgebiete.* Berlin. 1961, June, v. 7, No. 6, 472–4.

The English summary appended to the paper is as follows:—

"39 Salmonella belonging to 13 sero types were found in 35 Greek tortoises when the faeces of 52 clinically healthy tortoises earmarked for sale in two zoological shops of the city of Leipzig were examined."

VAN OYE, E. Sur la nature de l'antigène 0:27 des Salmonellae. [**The Nature of *Salmonella* 0:27 Antigen**] *Ann. Inst. Pasteur.* 1961, Apr., v. 100, No. 4, 463–9.

This article appears to be substantially the same as that presented in *C.R. Soc. Biol.*, 1960, v. 154, 1656 [see this *Bulletin*, 1961, v. 36, 935] by the same author, although no reference is made to the previous communication. Evidence is submitted to the effect that 0:27 is a thermolabile surface antigen and not a true somatic antigen such as 0:4. The lability of 0:27 is not of the same type as 0:5 but both should be considered as capsular antigens and not O antigens.

H. J. Bensted

KALLE, G. P. & GOTS, J. S. **Mechanism of Resistance to 2,6-Diaminopurine in *Salmonella typhimurium*.** *Biochim. et Biophys. Acta.* 1961, July 22, v. 51, No. 1, 130–37, 3 figs. [23 refs.]

ARMSTRONG, F. B. & WAGNER, R. P. **Biosynthesis of Valine and Isoleucine. IV. α-Hydroxy-β-Keto Acid Reductoisomerase of *Salmonella*.** *J. Biol. Chem.* 1961, July, v. 236, No. 7, 2027–32, 5 figs. [14 refs.]

KAMPELMACHER, E. H. & GUINÉE, P. A. M. **Four New *Salmonella* Types (*S. Tilburg, S. Bunnik, S. Overschie* and *S. Sterrenbos*) isolated in the Netherlands.** *Antonie van Leeuwenhoek: J. Microbiol. & Serol.* 1961, v. 27, No. 2, 193–5.

"Four new *Salmonella* types isolated in the Netherlands are described. *S. tilburg*, 1, 3, 19 ; d:l, w, was isolated from the lymph nodes of a swine. *S. bunnik*, 43 ; z42:—, was isolated from a reptile. *S. overschie*, 51; 1, v: 1, 5, was isolated from a tortoise. *S. sterrenbos*, 6, 8 ; d: enx, was isolated from the lymph nodes of a patient."

GUINÉE, P. A. M. & KAMPELMACHER, E. H. **Four New *Salmonella* Types (*S. Gambaga, S. Kumasi, S. Pramiso* and *S. Ashanti*) isolated in Ghana.** *Antonie van Leeuwenhoek: J. Microbiol. & Serol.* 1961, v. 27, No. 2, 203–5.

"Four new *Salmonella* types isolated in Ghana are described. *S. gambaga*, 21 ; z35: enz15, *S. kumasi*, 30 ; z10: enz15, and *S. pramiso*, 3, 10: c: 1, 7, were isolated from reptiles ; *S. ashanti*, 28 ; b: 1, 6, was isolated from a rat."

BREDE, H. D. *Salmonella Woodstock*—'n nuwe serotipe [16:Z_{42}:1,(5),7]. [**Salmonella Woodstock, a new Serotype (16:Z_{42}:1,(5),7)**] *South African Med. J.* 1961, July 8, v. 35, No. 27, 556.

The English summary appended to the paper is as follows:—

"A Gram-negative germ, isolated from the faeces of an 18-year-old woman, with the suggestive diagnosis of amoebic dysentery, was found to be a new serologica type of the salmonella group. (Chemical properties urea, —; dextrose, acid and gas ; mannitol, acid an gas ; saccharose, —; lactose, —; H_2S, +++; indol, – raffinose, —; inositol, —; dulcitol, acid and gas ; so bitol, acid and gas ; aesculin, only acid ; xylose, onl acid.)

"On serological examination *S. woodstock* wa strongly agglutinated only by the O-antiserum 16. Th motile germ was primarily agglutinable only by phase- antisera. It was impossible, either for us or th Salmonella Reference Laboratory, London, to obtai the specific phase 1. Dr. P. R. Edwards (Communicab Disease Center, Chamblee, Georgia) succeeded in grov ing phase 1 and, therefore, in determining the definit sero-formula: 16: z_{42}:1, (5), 7.

"In view of the fact that the patient probably cor tracted the disease in Woodstock, we named the ne type *Salmonella woodstock*.

"*S. woodstock* was primarily resistant to penicilli erythromycin, streptomycin and novobiocin. It wa sensitive to chloromycetin, kanamycin, neomycin, an all the tetracyclines."

MACKEL, D. C., LANGLEY, Lorreine F. & VENICE, L. **The Use of the Guinea-Pig Conjunctivae as a Experimental Model for the Study of Virulenc of *Shigella* Organisms.** *Amer. J. Hyg.* 1961, Ma v. 73, No. 2, 219–23.

[Although conjunctivitis and iridocyclitis are we recognized as toxic manifestations of bacillary dysente they have never been common complications of th disease. Even among the considerable number serious cases of Shiga dysentery seen in the Middle Ea during the First World War these eye complicatio were infrequent and generally restricted to the mo toxic cases. Conjunctivitis, a little more common, w seen in conjunction with arthritic complications of Shi dysentery ; it was occasionally seen also when rabb were injected with Shiga toxin.]

ZOELLER and MANOUSSAKI (*C.R. Soc. Biol.*, 1924, 91, 257) showed that local application of cultures Shiga's bacillus to the conjunctivae of rabbits previous treated with bile resulted in an inflammatory conditi of the eye, but how closely these observations wi Shiga's bacillus, which presumably were related to t specific exotoxin of this organism, can be connect with the keratoconjunctivitis reaction of SERENY [s this *Bulletin*, 1958, v. 33, 690; 1959, v. 34, 567; 19 v. 35, 191, 812], seems a little uncertain. [Most of t work on dysenteric keratoconjunctivitis in guineap stems from the original experiments of Sereny and th were more concerned with the mannite-fermenti dysentery organisms which did not produce a solu exotoxin. While the results of the experiments Sereny and his followers (see also MANOLOV, *ibid.*, 19 v. 33, 689) suggest that the conjunctival reaction p duced by various strains of shigellae when applied guineapigs eyes, is related to the property of the p ticular organism to produce clinical dysentery in th thus infected.] There have been several reports confirm the original observations of Sereny. T

rticle under review does not offer information that is undamentally new; in the main the evidence supports he findings of Sereny and his followers in various ommunications presented in papers from Central uropean sources.

[See also this *Bulletin*, 1959, v. 34, 969.]

H. J. Bensted

SERÉNY, B. **Impressions of *Sh. sonnei* Colonies on Agar Culture Media.** *Acta Microbiologica.* Budapest. 1960, v. 7, No. 3, 277–84, 5 figs. [21 refs.]

This author has already published accounts of the peculiar colonial morphology of *Shigella sonnei* Phase and Phase II [see this *Bulletin*, 1960, v. 35, 396, 928]. n the present paper he draws attention to some further bservations he has made, particularly with regard to Phase II colonies grown on yeast extract agar. When he colonies are removed *in toto* from plate cultures the nderlying surface of the agar shows formations which argely reflect the structure of the colonies which grew here and are termed "colony impressions". The mpression left by a pure Phase II colony appears as a ound whitish opaque spot, while the star-shaped colony, previously described in detail, leaves behind a star-haped impression which is easily recognizable. Colonies f pure Phase I *Sh. sonnei*, on the other hand, do ot leave behind any recognizable impression on the urface of the agar. It is considered that the colony mpressions left behind by Phase II cultures are asso-iated with the close adhesion of the bacterial cells orming Phase II colonies and the firm adhesion of the ormed colony to the surface of the agar. This does ot occur with pure Phase I colonies. [The results of he observations recorded here are of academic interest, ut how far they are of value in routine or epidemio-ogical investigations seems a little doubtful.]

H. J. Bensted

ABBOTT, J. D. & GRAHAM, J. M. **Colicine Typing of *Shigella sonnei*.** *Monthly Bull. Ministry of Health & Pub. Health Lab. Service (directed by Med. Res. Council).* 1961, Mar., v. 20, 51–8.

Colicine typing of *Shigella sonnei* was previously eported by ABBOTT and SHANNON in 1958 [see this *Bulletin*, 1958, v. 33, 689] and the present paper gives n account of typing by the same method of 1,319 ther strains of this organism. In addition to the 7 olicine types described in the original paper 8 other ypes (Types 3A, 7, 8, 9, 10, 11, 12 and 13) have now een distinguished and it has become possible, by the se of 9 indicator strains (*Escherichia coli* Row, *Sh. onnei* strain 17, 56, 56/56, R6, R1, 2/7, R5 and *Sh. chmitzi* [*Sh. dysenteriae* 2], NCTC 8218) to recognize 5 distinct types. Most of the strains examined came rom the area in which the authors were working Manchester) and it is noteworthy to record that only 0% of these strains were untypable, whereas of 68 trains originating from Southampton there were 47 trains which proved to be untypable.

H. J. Bensted

ERLANDSON, A. L., Jr., FISHER, M. W., GAGLIARDI, Leola A., PEARSON, Iris A. & WAISBREN, B. A. **Characteristics of Strains of *Escherichia coli* associated with Severe Infections in Adults.** *J. Infect. Dis.* 1961, Mar.-Apr., v. 108, No. 2, 189–94, 1 fig.

In studying the mouse virulence of a number of different strains of *Escherichia coli*, which included strains isolated from adults with severe non-enteric infections in addition to enteropathogenic strains from children with acute gastro-enteritis (O55; O111, etc.), it was found that the "adult" strains were generally more virulent for mice than the enteropathogenic strains. Intraperitoneal injection with mucin of 6 non-enteropathogenic adult strains showed the LD50 to be less than 10 bacterial cells, and 3 other such strains had an LD50 of 100 cells. In contrast only one of 6 enteropathogenic strains showed a mouse-virulence of the same order. Tests without mucin were not so striking, but again the average LD50 for the enteropathogenic strains was higher than for the adult non-enteropathogenic strains. The two groups of strains were not related antigenically.

[The significance of these findings seems a little doubtful since there is considerable variation in the mouse-virulence of different strains of *Esch. coli* whether they are of the enteropathogenic type or not, and this is evident in the authors' enteropathogenic strains where within strains of the same antigenic structure there were marked differences in the LD50 for mice in the virulence tests with mucin.]

H. J. Bensted

DARBARI, B. S. & SAXENA, B. P. **Biochemical Studies of *Escherichia coli* isolated from Cases of Urinary Tract Infection and their Sensitivity to Sulpha Drugs and Antibiotics.** *J. Indian Med. Ass.* 1961, Apr. 1, v. 36, No. 7, 283–6. [10 refs.]

The authors examined the biochemical reactions and haemolytic activity of 89 strains of coliform organisms isolated from the urine of patients with urinary tract infections, many of which were chronic and failed to respond clinically to the sulphonamides and antibiotics. Only about one-third of the strains could be classified as *Escherichia coli* and although 35 of 89 were haemolytic, the haemolytic activity does not appear to have been related to the type of organism according to the classification set out. The drug sensitivity tests are equally unhelpful in this respect. A high proportion of the total strains examined were resistant to the sulphonamides and, with the antibiotics tested, Achromycin [tetracycline] appeared to give the best results—9% of the strains were completely resistant, but almost 50% were highly sensitive to this antibiotic.

[The significance of this bacteriological exercise seems very doubtful since none of the findings can be identified with a particular type of organism or clinical infection; they are merely routine laboratory records.]

H. J. Bensted

SPRINGER, G. F., STEERS, E., DHANAMITTA, S., STINNETT, J. & GYORGY, P. **Protection of Mice against Lethal *Staphylococcus* Infection by *Escherichia coli* O_{86} Fractions.** *Science.* 1961, Aug. 4, v. 134, 335–6.

"Small doses of protein-lipopolysaccharides and lipopolysaccharides from *Escherichia coli* O_{86} prevent the death of mice infected intraperitoneally with a clinically virulent *Staphylococcus aureus.*"

STADTMAN, E. R., COHEN, G. N., LEBRAS, Gisele & DE ROBICHON-SZULMAJSTER, Huguette. **Feed-Back Inhibition and Repression of Aspartokinase Activity in *Escherichia coli* and *Saccharomyces cerevisiae.*** *J. Biol. Chem.* 1961, July, v. 236, No. 7, 2033–8, 4 figs. [22 refs.]

RICHARDS, N. & HINSHELWOOD, C. **Observations on the β-Galactosidase Activity of *Bact. lactis aerogenes (Aerobacter aerogenes).*** *Proc. Roy. Soc.* Ser. B. 1961, Aug. 15, v. 154, No. 957, 463–77, 8 figs. [11 refs.]

"When fully adapted cells of *Bact. lactis aerogenes* (*Aerobacter aerogenes*) are grown in an aerated medium containing lactose as sole carbon source, the activity of β-galactosidase per unit mass is constant during the greater part of the logarithmic phase, but rises rapidly towards the end, reaching a maximum soon after the onset of the stationary phase. Subsequently the activity as determined with intact cells falls slowly, while that determined with cells whose permeability has been increased by shaking with benzene remains almost constant for some hours. On re-inoculation of the cells into a new medium, the activity falls rapidly to the steady value characteristic of the logarithmic phase.

"The *p*H/activity curve of the enzyme shows that the changes are not associated with a compensation effect by which decreasing specific activity at the lower *p*H late in the growth cycle is balanced by an increase in the amount of enzyme formed. The rise, moreover, occurs in cultures in which the *p*H is kept constant.

"Measurements on cultures grown (*a*) in a stream of nitrogen, where the activity is higher throughout; and (*b*) a stream of oxygen, where the rise in activity does not occur at all, strongly suggest that oxygen starvation is a major cause of the rise. More lactose is metabolized in compensation for its less efficient utilization during the last phase where growth has become partially anaerobic.

"The enzyme activity per cell varies in a somewhat different way from the activity per unit mass, but there is little evidence to suggest that the activity per cell is a more fundamental quantity than the activity per unit mass."

COWAN, S. T., STEEL, K. J., SHAW, Constance & DUGUID, J. P. **A Classification of the *Klebsiella* Group.** *J. General Microbiol.* 1960, Dec., v. 23, No. 3, 601–12. [35 refs.]

176 non-motile, non-gelatin-liquefying strains of *Klebsiella* could on the possession of fimbriae and certain biochemical tests be divided into 6 distin groups. 4 of these are familiar: *K. aerogenes, pneumoniae, K. ozaenae,* and *K. rhinoscleromatis*; an 2 are new: *K. edwardsii* and *K. edwardsii* var. *atlanta* As well as designating type cultures of these ne groups, the authors propose new typical type cultu strains for *K. aerogenes, K. pneumoniae,* and *K. rhin scleromatis*—both the British and American cultur collection numbers are given for all these bacteria. table giving the salient differentiating characters of th six groups is given, which when used in the Determina tor [this *Bulletin,* 1960, v. 35, 903] will be helpful i identifying unknown klebsiellae. This grouping of th *Klebsiella* genus fits in with previous methods of divi ing the genus; there appears to be a relation betwee the serological type of the strain and the group int which it is put. *Elizabeth M. Harper*

UNITED STATES DEPT. HEALTH, EDUCATION & WELFAR **Studies on the Aeromonas Group** [EWING, W. H HUGH, R. & JOHNSON, Jane G.] 37 pp., 8 fig [Numerous refs.] 1961, Jan. Atlanta: Pub. Healt Service, Communicable Disease Center, Georgi U.S.A.

Aeromonas was the name given by KLUYVER and VA NIEL [this *Bulletin,* 1936, v. 11, 929] to a group c Gram-negative rods that resembled pseudomonads i being motile by polar flagella but differed by producin gas when sugars were fermented. In the last few yea much work has been done on the group [see EDD *J. Applied Bact.,* 1960, v. 23, 216; LYSENKO, se above p. 1181] and its definition improved. It is no agreed that the organisms attack carbohydrates b fermentation [as distinct from pseudomonads, whic oxidize them], and are oxidase-positive.

Ewing, Hugh and Johnson examined 138 strai which they regard as belonging to the group; 67 we classified as *Aeromonas hydrophila,* 21 as *A. salmon cida* (a pathogen of fish), and 50 as *A. shigelloide* The authors do not think it is profitable to subdivi these bacteria on the V-P reaction (*cf* Eddy). Of th species they recognize, *A. shigelloides* is important t the medical bacteriologist as most of the strai examined came from human faeces. The organism ha been known as C 27 and was first described by FERGU SON and HENDERSON [this *Bulletin,* 1948, v. 23, 355 It does not produce gas from sugars, and many strai share an antigenic relationship with *Shigella sonnei*; can, however, be distinguished from the shigella b motility.

The chief features that distinguish the three speci are shown in the table.

	A. hydrophila	*A. salmonicida*	*A. shigelloid*
Gas from Glucose	d	+	−
Lactose (acid)	d	−	+
Sucrose (acid)	+	−	−
Mannitol	+	+	−
Inositol	−	−	+
Indole	d	−	+
Gelatin	+	+	−
Motility	+	−	+

S. T. Cowan

SERPA, C. E. V. & FUKS, M. A. **Observations on Cross Reactions occurring between Tubercle Bacilli and Brucellae.** *Anais Microbiol.* Rio de Janeiro. 1960, v. 8, 169–79, 2 graphs. [20 refs.]

"Trying to clear up the nature of cross reactions observed in agglutination tests between sera of patients with tuberculosis and brucellosis we performed hem-agglutination reactions (Middlebrook & Dubos test) using polysaccharide antigen from *Brucella* and from tubercle bacillus on 45 sera from tuberculous patients and 25 sera from patients with brucellosis.

"Tuberculous sera gave positive reactions with polysaccharide antigen from *Brucella*; the opposite was also observed, *i.e.* sera from patients with brucellosis gave positive reactions with polysaccharide antigen from the tubercle bacillus.

"The number of these reactions and their intensity were, however, low."

WUNDT, W. Die Biochemie und Serologie der Brucellen. [**Biochemical Activity and Serology of the Brucellae**] *Ergebn. Mikrobiol. Immunitätsf.* 1961, v. 34, 120–79, 11 figs. [Numerous refs.]

A long review of the conflicting literature of the brucellae is presented based on more than 250 papers which are cited in ten pages of bibliography. Much of the material has already been reviewed in this *Bulletin*, especially the author's recent papers and those of PARNAS [WUNDT, this *Bulletin*, 1958, v. 33, 1043; 1959, v. 34, 42, 386; 1960, v. 35, 193, 304; PARNAS and KOZAKOWNA, *Arch. Inst. Pasteur de Tunis*, 1958, v. 35, 179; PARNAS, this *Bulletin*, 1959, v. 34, 334, 439; 1960, v. 35, 157; 1961, v. 36, 1068]. The general findings are most usefully summarized in tables 10 and 11, the first taken from Bergey's *Manual* and the second giving the reactions to be expected with the species and types differentiated in the first.

The complexity and difficulty of this work is illustrated on pages 135 and 136 by the observations of SCHUHARDT [this *Bulletin*, 1949, v. 24, 708; 1950, v. 25, 526] and his colleagues that tryptose recommended in the first report of the Expert Committee of the WHO as a source of N in *Brucella* media had actually become inhibitory of growth after 2 years' storage.

The rationale of the sodium diethyl dicarbonate test of RENOUX in which double and single rings of inhibition are obtained on inoculated plates around paper discs soaked with solution of this substance is discussed. It is suggested that the chelating agent may remove essential metal ions present as traces in the medium and thus inhibit growth, but that the reducing activity of the bacteria preventing oxidation of the compound may eliminate this effect. Even accepting such effects as probable the appearances obtained are not fully explained.

The general conclusion reached is that there is unanimity as to the bacteria that should be included in the *Brucella* group and also as to the best tests for making differentiations within the group, *i.e.* resistance to dyes, production of H_2S and serological reactions. There is not, however, a similar unanimity in classifying the three main forms, *melitensis, abortus* and *suis*, as separate species or merely as varieties.

J. W. McLeod

PHILIP, C. B. & OWEN, Cora R. **Comments on the Nomenclature of the Causative Agent of Tularemia.** *Internat. Bull. Bact. Nomencl. & Taxon.* 1961, July 15, v. 11, No. 3, 67–72. [21 refs.]

The organism that causes tularaemia has been placed in many different bacterial genera; *Bacterium, Pasteurella, Coccobacillus, Eucystia, Brucella, Francisella*, and *Tularecella*. Philip and Owen discuss the validity of these generic names and the taxonomic wisdom of including the tularaemia organism in different genera. They think that the organism should be excluded from *Pasteurella* and *Brucella*, the two most popular assignations, and can be correctly made the type of the genus *Francisella*, as proposed by DOROFEEV in 1947 (Symposium of research workers of Inst. Epidemiol. Mikrobiol. in Chita, 1947, v. 1, 177). *S. T. Cowan*

MERRIOTT, J., SHOEMAKER, Alice & DOWNS, Cora M. **Growth of *Pasteurella tularensis* in Cultured Cells.** *J. Infect. Dis.* 1961, Mar.–Apr., v. 108, No. 2, 136–50, 9 figs. [23 refs.]

The strains of *Brucella tularensis* used in the experiments recorded in this paper included a clone of the classical Schu strain designated SmS4 which was highly virulent for rabbits, mice and guineapigs; the LD50 was 2–4 organisms. Other strains were not so highly virulent or the LD50 for the three experimental animals not the same.

The cells used for the tissue cultures were the L strain of mouse fibroblasts, bovine kidney cells and human amnion cells. Various media were used for the cell cultures with different types and different concentrations of sera. All the cells grew readily on glass and when adequate growth was obtained the cells were trypsinized, washed, counted and then seeded into bottles or tubes containing cover slips and the appropriate growth medium in suitable numbers.

As in similar studies with other organisms [this *Bulletin*, 1957, v. 32, 612; 1958, v. 32, 609, 1176] it was found that highly and moderately virulent strains of *Br. tularensis* were ingested by, and multiplied within, the mammalian cells cultured *in vitro*, but that avirulent strains failed to grow intracellularly even when heavy doses of such organisms were added to cell cultures. The degree of intracellular multiplication was directly related to the virulence of the strain of organism being cultured; the multiplication of the highly virulent strain SmS4 was equally rapid in all types of culture cells used. Moreover the initial virulence of the SmS4 strain was maintained through 9 subcultures in mouse L cells. Slight cytopathogenicity resulted from the intracytoplasmic multiplication of *Br. tularensis* in the mouse L cell, but the growth pattern of the organism was considerably influenced by the type and amount of serum and by the nutrients in the growth medium.

H. J. Bensted

Huziwara, T., Watanabe, Y. & Hyôdô, S. Phagocytose *in vivo* de *Past. tularensis* par les leucocytes de la souris. Relations entre phagocytose et virulence de bactérie. [**Phagocytosis of *Brucella tularensis in vivo* by Leucocytes of Mice; Relationship between Phagocytosis and Virulence**] *C.R. Soc. Biol.* 1960, v. 154, No. 11, 2169–72, 3 figs.

Burrows and Bacon [*Trop. Dis. Bull.*, 1954, v. 51, 794], working with *Pasteurella pestis*, showed that virulent organisms which had been grown in the peritoneal cavity of the mouse, unlike organisms grown on artificial medium, developed the power to resist phagocytosis, while organisms of an avirulent strain did not. These authors, in similar experiments, but using *Brucella tularensis*, failed to obtain these results. With them, of the organisms grown on artificial medium (that of Hiwatasi), the virulent strain was more susceptible to phagocytosis than the avirulent strain. When organisms grown *in vivo* were tested, those recovered from the spleen reacted like the artificially grown organisms, while those recovered from the peritoneal cavity behaved like the avirulent strain.

D. A. Cannon

Pirt, S. J., Thackeray, E. J. & Harris-Smith, R. **The Influence of Environment on Antigen Production by *Pasteurella pestis* studied by means of the Continuous Flow Culture Technique.** *J. General Microbiol.* 1961, May, v. 25, No. 1, 119–30, 4 figs. [18 refs.]

[An abstract of this paper appeared in the *Tropical Diseases Bulletin*, 1961, v. 58, 1237.]

Smith, D. A. **Absence of Lysogeny in *Pasteurella pestis*.** [Correspondence.] *Nature.* 1961, July 29, v. 191, 522–3. [10 refs.]

Huziwara, T., Hyôdô, S. & Watanabe, Y. Effet d'antibiotiques sur la tularémie expérimentale chez la souris. [**The Action of Antibiotics on Tularaemia in the Mouse**] *C.R. Soc. Biol.* 1960, v. 154, No. 10, 1895–7. [14 refs.]

Streptomycin inhibits the growth of *Brucella tularensis* with test-tube cultures of the organism, but in tissue culture the antibacterial effect of streptomycin on *Br. tularensis* is slight, although the drug has proved to be a satisfactory therapeutic agent for the treatment of tularaemia. In the case of other antibiotics, such as oxytetracycline, chloramphenicol and erythromycin, their antibacterial effect on the growth of the organism is equally good whether in tissue culture or on ordinary laboratory culture media. It is suggested that the difference between the effect of streptomycin and the other antibiotics on the intracellular growth of *Br. tularensis* depends on the different cell-penetrating powers of the drugs.

In experimental tularaemia in mice, however, the picture is different. Streptomycin is almost as good a therapeutic agent as oxytetracycline and better than chloramphenicol or erythromycin. In comparative tes between streptomycin and oxytetracycline with grou of mice with experimental tularaemia it was found th when recovered mice were killed about one month aft infection a small number of the streptomycin-treate animals were still harbouring *Br. tularensis*; there wa no such evidence in the oxytetracycline-treated mice. would therefore appear that streptomycin is not suc an efficient therapeutic agent as oxytetracycline, but i view of the penetrating ability of chloramphenicol i the tissue culture test it is difficult to explain the rel tively poor results of this drug in the experiment infections in this trial. *H. J. Bensted*

Hirsch, H. A. & Paine, T. F., Jr. **Experimenta Uterine Tetanus in Mice.** *J. Path. & Bact.* 196 July, v. 82, No. 1, 195–8.

"Tetanus spores alone did not produce tetanus aft subcutaneous or intra-uterine injection in mice.

"Calcium chloride, certain bacterial species, an mouse embryonic tissue were effective adjuvants in pr moting tetanus infection.

"Tetanus spores injected together with an adjuva into the non-pregnant mouse uterus produced tetan about half as frequently as tetanus spores injected sub cutaneously.

"The implantation of spores into the mouse uter after abortion had been induced by the insertion of thread or by the intramuscular injection of diethy stilboestrol led to tetanus in every instance.

"Tetanus spores were still viable 2–6 weeks aft subcutaneous or intra-uterine injection and could b activated by calcium chloride or by *Staphylococcu aureus* injected at the site of the previous spo implantation."

Millo, A. & Scatozza, F. Sul comportamento del colinesterasi nel plasma e nei tessuti nervoso muscolare di cavie tetanizzate. [**Behaviour Cholinesterases in Plasma and Nervous an Muscular Tissues of Guineapigs Treated wit Tetanus Toxin**] *Giorn. di Microbiol.* Naples. 196 Jan.–Mar., v. 9, No. 1, 24–32, 1 fig. [25 refs.]

The English summary appended to the paper is follows:—

"The authors carried out some researches with th purpose of studying the behaviour of the cholinesterase of plasma and nervous and muscular tissues of guine pigs treated with tetanus toxin.

"From the obtained results they observe that the tru cholinesterase and pseudocholinesterase activity appea to be reduced in the sciatic nerves since the first stag of intoxication. So far as the spinal cord is concerne the true cholinesterase concentration increases at th beginning of intoxication and shows normal valu thereon while the pseudocholinesterase does not chan significantly. Cholinesterase activity of plasma, gastr cnemius muscle and cerebral cortex appears unchange as compared with control animals. Moreover, th authors observed an enlargement and softening of th sciatic nerve with an increase of its water content.

"They conclude that tetanus toxin interferes on chemical mediators of the nervous impulse. Nevertheless it has to be shown if the decrease of cholinesterase activity of the sciatic nerve represents the primary biochemical lesion or a consequence of the anatomical modifications observed macroscopically."

HILDEBRAND, G. J., LAMANNA, C. & HECKLY, R. J. **Distribution and Particle Size of Type A Botulinum Toxin in Body Fluids of Intravenously Injected Rabbits.** *Proc. Soc. Exper. Biol. & Med.* 1961, June, v. 107, No. 2, 284–9.

"In rabbits which were given type A botulinum toxin intravenously, and which died rapidly of acute poisoning, the toxin was found in lymph and plasma with no tendency to diffuse into erythrocytes. Ratio of toxin in lymph to plasma ranged between 1:6 to 1:12. Sedimentation coefficients of the toxin in these body fluids were similar and had the dimensions of a protein. The particle size was decreased in relation to crystalline toxin, was similar to toxin in lymph from rats given toxin intraduodenally, but was not sufficiently smaller to warrant the assumption of complete dissociation of toxin from the hemagglutinin present in crystalline toxin.

"Toxin was absent from fetal blood, but appeared in small quantities in urine and bile. Cerebrospinal fluid was free of toxin except for a small amount at death. In the normal eye none or only a small quantity of toxin appears in the intraocular fluid. By experimental reduction of intraocular pressure toxin in intraocular fluid tends to approach concentrations present in plasma. Inflammation can lead to the occurrence of toxin in intraocular fluid. The findings are compatible with the hypothesis that the toxin *in vivo* has the dimensions of protein and passes tissue barriers by filtration mechanisms normally operative."

COLLEE, J. G. **The Nature and Properties of the Haemagglutinin of *Clostridium welchii*.** *J. Path. & Bact.* 1961, Apr., v. 81, No. 2, 297–312, 1 fig. [29 refs.]

The author has investigated the production, by *Cl. welchii* cultures of haemagglutinin for human group A blood, of an enzyme destroying the receptors of myxoviruses on the red blood cells, and the "enzyme" which removes the specific antigens characteristic of the different blood groups. He accepts WICKHAM's conclusion that the agglutinin is diffusible but cannot accept her suggestion that these three properties depend on a single substance. The fact that the agglutinin can act at 4°C., and is not eluted at 37°C. makes it unlikely that the substance is an enzyme. Furthermore, inasmuch as the agglutinin is not easily recognized in freshly isolated cultures but has been demonstrated only in old stock cultures, it may be a degraded form of enzyme normally active on cell surface polysaccharides. In this connexion it is interesting that the best yield of diffusible haemagglutinin was obtained from cultures in Ellner's medium in which a large proportion of sporing forms develop. [Wickham, see this *Bulletin*, 1956, v. 31, 867.]

Attention is drawn to an inhibitor (C) of this agglutinin present in cooked meat broth and distinct from the inhibitor (A) of the A-group isoagglutinin which can be isolated from peptone.

The (C) inhibitor is capable of preventing agglutination of blood by myxoviruses and it is destroyed by the RDE of *V. cholerae* culture filtrates.

J. W. McLeod

SAPUPPO, A. Sull'azione del lisozima sul treponemo pallido. [**Action of Lysozyme on *Treponema pallidum***] *Nuovi Ann. d'Igiene e Microbiol.* 1961, Jan.–Feb., v. 12, No. 1, 72–8, 4 figs.

The English summary appended to the paper is as follows:—

"The lysozyme activity on pathogenic Treponema pallidum (Nichols' strain) has been studied. The motility of living micro-organisms was reduced and cytologic alterations were demonstrated; dead or nonmotile Treponemata were strongly agglutinated. The agglutination phenomena appear very remarkable with old suspensions."

BERTÓK, L. & KEMENES, F. **Studies on the Lipase-System of Leptospirae. I. Tributyrinase Activity.** *Acta Microbiologica.* Budapest. 1960, v. 7, No. 3, 251–9, 8 figs. [10 refs.]

The authors found that lipase (tributyrinase) production in cultures of lipolytic leptospires increased proportionately to the growth of the organisms (*Leptospira icterohaemorrhagiae* and *L. pomona*). In cultures of *L. pomona* lipase appeared earlier, and persisted longer, than haemotoxin; the activity of both factors persisted after death of the organisms and neither factor could be dialysed; but lipase was comparatively thermostable up to temperatures of 60°C., unlike haemotoxin, whose activity decreased markedly at temperatures over 40°C.

Cultures of pathogenic and saprophytic leptospires also hydrolysed natural oils (olive, sunflower, sesame, castor and linseed).

Tributyrinase activity of saprophytic leptospires (3 strains of *L. biflexa*) was found to be greater than that of strains of pathogenic serotypes (*icterohaemorrhagiae, canicola, pomona, grippotyphosa, sejroe* and *saxkoebing*, and *hyos*) some of which were virulent, some avirulent, and some intermediate. But avirulent and moderately virulent strains of the first 3 serotypes showed more activity than virulent strains of the latter 3 serotypes.

After prolonged maintenance in culture, pathogenic leptospires lost virulence, lipase activity and haemotoxic activity; by contrast, the saprophytes retained lipase activity. However, production of lipase and haemotoxin cannot be related to virulence in the case of *L. pomona* (strain Nagymágócs 1953); this is unable to multiply in animals but culture filtrates inoculated intravenously into young lambs regularly cause haemoglobinaemia, haemoglobinuria, jaundice, and death.

The rate of leptospiral lipase reaction can be decreased by inhibitors (sodium arsanilate, neoarsphenamine, sodium fluoride) and increased by cysteine HCl which can also antagonize the inhibitory effect of

sodium ansanilate: these phenomena may depend on the number of SH groups in the protein molecule of the lipase and the effects of the inhibitors on these groups.

It is possible that differences in lipase activity between strains of the same serotype (which are antigenically identical) may account for differences in their pathogenicity: for example, *L. bataviae* in Italy causes a mild illness whereas strains in South East Asia cause Weil's syndrome and death. *L. H. Turner*

STURDZA, Nina & ELIAN, M. **Comparative Study on Different Strains of *L. biflexa* as Antigen for the Complement Fixation Test in Leptospirosis.** *Arch. Roumaines Path. Expér. et Microbiol.* 1961, Mar., v. 20, No. 1, 33–41. [13 refs.]

Earlier work suggested that an antigen obtained from *Leptospira biflexa* (Patoc strain) was useful in a complement-fixation test (CFT) for leptospirosis which could be carried out by any routine diagnostic laboratory.

The authors now report on the comparison of such CFT antigens which were prepared from 4 strains of the saprophyte, *L. biflexa* (Patoc, Madida, Peschiera, Ancona-Porto) and from the pathogen *L. semarang* (which, with Madida, is antigenically related to Patoc, on a basis of cross-absorption reactions).

These CFT antigens were tested against immune rabbit sera prepared from 27 other pathogenic serotypes and the 5 homologous leptospires as well as 2 normal controls and one anti-salmonella para-C rabbit serum. None of the antigens reacted with the 3 control sera. The number of "pathogenic" antisera with which the various antigens reacted (titres of 1/8 or higher) were: Patoc, 24/28; Peschiera, 21/28; Ancona-Porto, 13/28; Madida, 11/28; *L. semarang*, 8/27. Most (22/28) of the immune "pathogenic" sera had been stored at + 4°C. for 1–3 years which may have resulted in a fall in CFT titre; but Patoc failed to react with recently prepared sera against *L. icterohaemorrhagiae* AB and *L. australis* B of which the agglutination (ALT) titres were 1/12,800 and 1/4,000.

The antigens were also tested against 30 human sera positive by ALT in which the maximum titres were for the following serotypes: *pomona* and *sejroe*, 10 each; *hyos*, 2; *icterohaemorrhagiae, bataviae, grippotyphosa, poi*, 1 each; and 4 sera reacted with 2 antigens to the same titre. The numbers of these sera which reacted positively with the test antigens were—Patoc 29, Madida 18, *semarang* 15, Peschiera 10, Ancona-Porto 2.

These findings confirm that antigen derived from *L. biflexa* Patoc is the most useful. *L. H. Turner*

BAUER, D. C., EAMES, L. N., SLEIGHT, S. D. & FERGUSON, L. C. **The Significance of Leptospiral Hemolysin in the Pathogenesis of *Leptospira pomona* Infections.** *J. Infect. Dis.* 1961, Mar.–Apr., v. 108, No. 2, 229–36, 3 figs. [14 refs.]

The authors prepared a test haemolysin derived from the precipitate obtained by treating culture filtrate of *Leptospira pomona* with ammonium sulphate (31–35% saturation): their final product was 4 times as active as culture filtrate.

Haemolysin was administered intravenously to lambs aged 2–4 weeks: 2 of these lambs showed 30–50% reduction in packed cell volume (PCV) and Hb values but paleness of mucosae was the only sign both recovered, and PCV and Hb values were normal by the 10th day. The other 2 lambs showed 60–80% reduction in PCV and Hb within 24 hours, weakness, icterus, and haemoglobinuria; and at 31 hours one lamb died, the other was killed.

Autopsies performed during the acute phase of anaemia revealed dark red urine, and generalized icterus most marked in liver, mucosae and skin. The main histopathological findings were areas of centrilobular necrosis in the liver and a homogeneous red material in the kidney tubules—the nuclei of which were pyknotic. No abnormalities were found in the brain, heart, adrenals, or in skeletal muscle.

Haemolysin had no effect on 2 lambs with *L. pomona* serum agglutinins (titres 10^{-2}, 10^{-5}). Repeated injections of sublethal amounts of haemolysin failed to cause an increase in haemolysin—inhibition titre of the sera.

A suspension of washed *L. pomona* (10 ml.; 10– organisms per ml.) was injected intravenously into lambs to observe the haemolytic processes *in vivo*. Haemolysis was associated with the appearance of leptospires in the plasma and continued for 2 days in the presence of a rising agglutinin titre (although leptospires disappeared). It seemed that the haemolytic process was irreversibly established before antibody was produced, a view supported by 2 *in vitro* experiments which showed that (1) haemolysin is adsorbed non-specifically (and also on to sheep cells, guineapig cells and polystyrene latex), and (2) once haemolysin is adsorbed on to red cells, the inhibitory effect of anti-serum is diminished.

The precise nature of the haemolysin and its mode of action have yet to be defined; but it seems that the jaundice produced by some strains of leptospires is haemolytic in origin (other strains cause obstruction of the bile ducts and impaired liver function as a result of destruction of hepatic cells).

[For earlier work on leptospiral haemolysins see this *Bulletin*, 1956, v. 31, 953; 1957, v. 32, 998; 1959, v. 34, 577; and KEMENES, *Acta Veterinaria, Belgrade*, 1958, v. 8, 143]. *L. H. Turner*

MARTINEC, T. & KOCUR, M., with the technical assistance of Z. VYSLOUZILOVÁ. **A Taxonomic Study of the Members of the Genus *Serratia*.** *Internat. Bull. Bact. Nomencl. & Taxon.* 1961, July 15, v. 11, No. 3, 73–8. [14 refs.]

BACTERIOPHAGES

BARRICELLI, N. A. & DOERMANN, A. H. **An Analytical Approach to the Problems of Phage Recombination and Reproduction. III. Cross Reactivation.** *Virology*. New York. 1961, Apr., v. 13, No. 4, 460–76, 7 figs. [11 refs.]

SOMPOLINSKY, D. [**A New Bacteriophage useful in Typing**] *Harefuah*. Jerusalem. 1961, Aug. 1, v. 61, No. 3, [in Hebrew 100–102. English summary 102].

The English summary appended to the paper is as follows:—

"An account is given of a new staphylophage, which has provisionally been called *phage Israel*.

"More than 25% of *Staphylococcus aureus* strains, which caused human infections in Israel, were lysed only by this phage. The frequence was about 10% in carrier strains from hospital staff, and only 3% in carrier strains from the general population. In veterinary strains this type is very rare.

"The phage is serologically related to the group A phages, and its lytic spectrum is related to those of phages 80 and 81. The author suggests that staphylococcus strains lysed only by this phage may be a local 'epidemic' type."

FREDERICQ, P. & GRATIA, J. P. Recherches génétiques sur la résistance croisée au phage T1 et à la colicine B chez *Escherichia coli*. [**Genetic Research on Cross-Resistance to Phage T1 and Colicine in *E. coli***] *C.R. Soc. Biol.* 1960, v. 154, No. 11, 2150–53.

Some bacterial receptors appear to be shared by colicines and phages; if the receptor is lost the bacteria acquire resistance to both colicine and phage simultaneously. However, some mutants of *Escherichia coli* K12 which are resistant to phage T1 show only partial cross-resistance to colicine B. To investigate this relationship the authors have isolated a number of mutants resistant to phage T1 or to colicine B and they have examined their sensitivity to phages T1 and T5 and to colicines B, V and I, their nutritional requirements and, by recombination experiments with F^+ and F^- mutants of the various types, they have localized the different mutations on the bacterial chromosome.

The mutants were of 5 types. Type (1) Prototrophic mutants resistant to both phages T1 and T5 and partially resistant to colicine B arising as the result of a chromosome deletion between markers Az and Lac near TL. Types (2), (3) and (4) resulted from allelic mutations which occurred in the region between markers Gal and CH. Type (2) mutants were prototrophs resistant to T1, but not to T5, and totally resistant to colicines B, V and I. Type (3) mutants resembled type (2) except that they were tryptophan-requiring. Type (4) mutants were prototrophs resistant to colicines B and I but without cross-resistance to phages T1 and T5. Type (5) mutants, like type (1), resulted from a deletion but in the region of the chromosome between markers cystine and streptomycin. They were totally resistant to colicine B, partially resistant to colicine I, sensitive to both phages and methionine-requiring. (This mutation occurred in a region distinct from the locus already known to control methionine requirements.)

Thus resistance of *E. coli* K12 to colicine B is controlled by 3 distinct loci, 2 of which also determine resistance to phage T1. The authors conclude that the synthetic pathways in the bacteria which lead to the formation of the 2 kinds of receptor are not completely identical but overlap. *Janice Taverne*

MINAGAWA, T. **Some Characteristics of the Internal Protein Phage T2.** *Virology*. New York. 1961, Apr., v. 13, No. 4, 515–27, 6 figs. [10 refs.]

T2 phage contains an acid-insoluble internal protein comprising about 7% of the total protein [HERSHEY, this *Bulletin*, 1958, v. 33, 397] and also an internal complement-fixing antigen which is independent of the protein coat [LEVINE *et al.*, *ibid.*, 1959, v. 34, 497]. When T2 lysates were purified, and the particles were disrupted by osmotic shock and sedimented in the ultracentrifuge, both internal protein (measured by its radio-sulphur content) and the complement-fixing property sedimented with the phage DNA in low salt concentrations; both dissociated from the DNA and remained in the supernatant fluid at concentrations greater than 0·1M NaCl or 0·01 $MgCl_2$. Synthesis of internal protein began immediately after infection; its rate reached a maximum by 10 minutes and then it fell to a constant level which was equal to the rate of production of phage particles. Like other proteins, its synthesis was inhibited by chloramphenicol. Labelled internal protein of infecting phage particles did not reappear as internal protein in the offspring.

No evidence of association of internal protein with phage DNA was obtained. In fact, as described (1) they dissociated at higher electrolyte concentrations, (2) DNA synthesis continued independently in the presence of chloramphenicol, and (3) DNA is transferred to progeny phage particles while internal protein is not. *Janice Taverne*

LEVINE, M. **Effect of Mitomycin C on Interactions between Temperate Phages and Bacteria.** *Virology*. New York. 1961, Apr., v. 13, No. 4, 493–9. [28 refs.]

Mitomycin C selectively inhibits DNA synthesis in *Escherichia coli* but synthesis is restored if the bacteria are then infected by phages T2, T3 or T5 (SEKIGUCHI and TAKAGI, *Biochim. et Biophys. Acta*, 1960, v. 41, 434). Similarly, a concentration of 10 μgm./ml. of mitomycin C was found to inhibit synthesis of DNA in *Salmonella typhi-murium* LT-2 without affecting RNA or protein synthesis, the cells enlarging but not dividing,

and inhibition was reversed by infection with phage P22. Infected cells gave rise to infective phage after a normal latent period, but the yield was halved and the phage obtained transduced with a frequency 10 times less than that of normal lysates. Mitomycin C prevented the establishment of lysogeny; all infected cells lysed in conditions where 90% of untreated cells became lysogenic. Mitomycin C did not affect the frequency of recombination obtained in mixed infection experiments. It had an inducing action on lysogenic bacteria, apparently similar to that of UV light since it caused induction of *Salm. typhi-murium* LT2 carrying prophage P22*c*$^+$, which is UV-inducible, but did not with *E. coli* C carrying prophage P2*c* which is not inducible by UV light.

Theoretically it is possible that the action of mitomycin C is reversed by phage infection because DNA synthesis then proceeds along an alternate pathway. It is also possible that phage DNA is synthesized from pre-formed components of bacterial DNA, so that if mitomycin C inhibited DNA synthesis at an early step, synthesis might resume upon infection but would lead to a lower yield of phage particles. *Janice Taverne*

VAN ARKEL, G. A., VAN DE POL, J. H. & COHEN, J. A. **Genetic Recombination and Marker Rescue of Urea-Disrupted Bacteriophage T4 in Spheroplasts of *E. coli*.** *Virology*. New York. 1961, Apr., v. 13, No. 4, 546–8.

EDGAR, R. S. & EPSTEIN, R. H. **Inactivation by Ultraviolet Light of an Acriflavine-Sensitive Gene Function in Phage T4D.** *Science*. 1961, Aug. 4, v. 134, 327–8, 2 figs.

UETAKE, H. & HAGIWARA, S. **Genetic Cooperation between Unrelated Phages.** *Virology*. New York. 1961, Apr., v. 13, No. 4, 500–506. [21 refs.]

Salmonella cells of group E_1 carry antigens 3 and 10, which upon lysogenization with phage ε^{15} are converted to 3, 15 (group E_2) [this *Bulletin*, 1956, v. 31, 74]. When these cells are then lysogenized by a second phage, ε^{34}, the antigens are converted to (3) (15) 34 (group E_3). However, lysogenization of *Salm. anatum* 293 (group E_1) by phage ε^{34} alone did not lead to any change in its antigens 3 and 10, and did not result in the appearance of antigen 34. Production of antigen 34 could not be stimulated by UV irradiation, and it appears that its synthesis plays no essential role in the production of infective phage. Apart from the fact that these cells were now lysogenic for phage ε^{34}, the only difference observed in them was a loss of sensitivity to phage C_{341}. When the cells were secondarily infected with phage ε^{15}, both antigens 15 and 34 appeared 10 minutes after infection. Antigen 34 was never formed without antigen 15, and its production apparently depended upon the presence of the machinery for synthesis of antigen 15. Thus, phenotypic expression of properties arising from lysogenic conversion do not inevitably follow the entry of the phage. As the authors point out, lysogenization by the second phage, ε^{15}, alone might have been considered the cause of the formation of both antigens 15 and 34 had they not known that the cells carried prophage ε^{34}. *Janice Taverne*

NORTHROP, J. H., with the technical assistance of MARIE KING. **Factors controlling the Production of Lysogenic Cultures of *B. megatherium*.** *J. General Physiol.* 1961, May, v. 44, No. 5, 859–67, 3 figs. [19 refs.]

In some experiments with T phage of *Bacillus megatherium* 899 the fate of infected cells was decided by the culture medium in which they were suspended after adsorption. Infected cells suspended in a yeast-extract-peptone medium mostly lysed, only 30 lysogenic colonies surviving from 10^7 cells. On the other hand, cells suspended in a medium containing asparagine gave rise to 10^6 lysogenic colonies per 10^7 infected cells. The addition of peptone or hydrolysed casein to the asparagine medium decreased the amount of lysogenization, as did a number of individual amino acids, but the presence of no one amino acid inhibited all lysogenesis. *Janice Taverne*

STRAUSS, B. S. **Specificity of the Mutagenic Action of the Alkylating Agents.** [Correspondence.] *Nature*. 1961, Aug. 12, v. 191, 730–31, 1 fig.

VIRUSES

The order in this section is:—**General; Psittacosis-Lymphogranuloma group; Pox group; Respiratory group; Reovirus group; Enteric group; Arthropod-borne group; Others.**

POLLARD, Morris [Edited by]. **The Gustav Stern Symposium on Perspectives in Virology. Vol. II.** pp. x + 220, illustrated. 1961. Minneapolis 15: Burgess Publishing Co., 426 South Sixth Street, Minn., U.S.A. [$8.00.]

This symposium, in honour of Dr. Peyton ROUS, was held in New York during January 1960. There are 15 chapters, each followed by an edited record of discussion.

T. M. SONNEBORN (pp. 5–12) describes the kappa particles of *Paramecium aurelia*; he regards these particles as intracellular parasites which vary in their pathogenicity. It was suggested in discussion by J. E. SMADEL that the kappa particles resemble the rickettsiae.

R. C. WILLIAMS (pp. 18–35) reviews the structure an

localization of virus-like particles within infected cells which are revealed by electron microscopy. A promising technique is the use of specific viral antibody coupled with an electron-dense molecule. In a later chapter, Françoise Haguenau, H. L. Febvre and Jacqueline Arnoult (pp. 160–170) describe the ultrastructure of Rous sarcoma virus growing in tissue cultures prepared from chick embryos. They describe intracytoplasmic particles which are either enclosed in a vacuole or embedded in a matrix.

G. Schramm (pp. 38–44) discusses ways of producing virus mutants by chemically altering their nucleic acid. At present, the most promising method is to treat RNA viruses with nitrous acid: mutants of tobacco mosaic virus and of poliovirus have been obtained.

W. W. Ackermann (pp. 45–55) has studied the cytoplasmic infection of HeLa cells after exposure to high multiplicities of vaccinia virus. Multiplication of the virus stimulates the synthesis of materials in the cytoplasm which do not constitute part of the viral body; in contrast, the nucleus undergoes a profound, but reversable, contraction.

H. S. Ginsberg (pp. 58–66) describes the infection of HeLa cells with the DNA adenoviruses. In this case, masses of DNA and protein accumulate in the nucleus of the cells, and again some of the newly synthesized material is not a part of the viral body.

In human adenoid tissue, the adenovirus-infected cell has a remarkable ability to retain its identity as a functional and physical unit. R. W. Schlesinger (pp. 69–77) considers some of the factors which may be important in permitting this high degree of compatibility; and he describes some conditions which cause the virus to destroy the cell.

J. E. Darnell, Jr. (pp. 80–87) is interested in the time lag in virus production which is observed when HeLa cells are infected with poliovirus. With the T-even bacteriophages, the delay can be explained by the time required for the synthesis of new enzymes, but in the poliovirus-infected cell the delay is more likely to be due to a lag in transport within the cell, perhaps the transport of viral RNA into the nucleus.

A. B. Sabin (pp. 90–108) describes the changes in the properties of polioviruses which are cultivated at temperatures within the range 25°–41°C. Neither the temperature nor the *d* marker can be regarded as virulence markers.

S. A. Waksman (pp. 111–116) views with philosophic detachment the failures experienced in the search for antiviral agents. He is followed by A. Isaacs (pp. 117–123) who describes some of the properties of interferon which may affect virus multiplication by stimulating the glycolytic activity of the cell.

R. M. Chanock, J. A. Bell and R. H. Parrott (pp. 126–138) discuss the natural history of parainfluenza infections. The average adult is said to experience 6 respiratory infections each year and if every episode represents infection with a distinct agent, then about 400 agents must be postulated. However, to take one example, parainfluenzae 3 can infect the same person 2 or more times.

S. Kibrick (pp. 140–157) reviews viral infections of the foetus and newborn: there is an interesting section on cytomegalic inclusion disease.

W. P. Rowe, R. J. Huebner and Janet W. Hartley (pp. 177–190) discuss the ecology of polyoma virus. They emphasize that there is a great need for data on the natural history and epidemiology of supposed tumour viruses, because the isolation of a tumour virus from a spontaneous tumour does not prove causation. The final chapter records an informal seminar on criteria for the identification of human oncogenic viruses. J. T. Syverton (pp. 195–198) defines an oncogenic virus as, (i) a virus that initiates an infectious process which *may* result in a tumour, or (ii) a virus which makes a cell malignant by virtue of infection of the cell. If only the first sense is implied it is better to talk about "proliferative" viruses.

In a short epilogue, R. J. Dubos (pp. 200–205) draws attention to the creative effects of infections by viruses in contrast to their destructive effects.

This book is excellently printed by what the reviewer supposes to be photo-litho offset. The photographs are also surprisingly well reproduced by this process.

Forrest Fulton

Smith, S. E. **A Maintenance Medium for Tissue Culture Virus Studies.** *Brit. J. Exper. Path.* 1961, June, v. 42, No. 3, 232–5.

A maintenance medium for tissue culture work is described, consisting essentially of a liver digest ultrafiltrate in balanced salt solution. 250 gm. of fresh horse liver are blended in 1000 cc. of 0·05 N HCl; 250 mgm. of pepsin powder are added and the mixture is incubated for 24 hours with the addition of 2·5 cc. of chloroform as a preservative. The whole is then boiled to destroy enzymes and precipitate protein, adjusted to *p*H 7·0 and freed from solids by filtration through cloth. For purposes of storage the digest may be sterilized by Seitz filtration. Before use it must be ultrafiltered. A collodion membrane is formed on the outside of a Berkefeld filter candle, and the digest is drawn into the filter by suction, the procedure being carried out in a refrigerator in order to reduce bacterial contamination. The tonicity is then adjusted to equality with normal saline, which can be done with sufficient accuracy by determining the dilution of each which will haemolyse red cells, followed by addition of the requisite amount of water. The *p*H is then finally adjusted to 7·0 and the product is sterilized by Seitz filtration; it has a shelf life of 1 month or more at 4°C. The total nitrogen content of a number of batches ranged from 2·65 to 3·31 gm. per litre, with proportions of amino-nitrogen of 51·4 to 56·0%. The maintenance medium consists of 10% liver ultrafiltrate in Earle solution, and may be used with primary monkey, dog, lamb, rabbit and pig kidney cell cultures.

D. J. Bauer

Lehmann-Grube, F. **Preparation of Cell Cultures from Human Amniotic Membranes.** *Arch. f.d. gesamte Virusforschung.* 1961, v. 11, No. 2, 258–75. [33 refs.]

"The preparation and growth of human amnion cells for production of cell cultures have been investigated. 1. There is wide variation in the yields of cells from

different amnions, and also in the proportion of cells adhering to the glass. 2. A relatively large number of the specimens are contaminated with yeast and molds. 3. Amnion cells spread rather than multiply *in vitro*. 4. Human serum is superior to calf or horse serum. 5. The concentration of human serum can be reduced to 10% by use of Eagle's medium. 6. Chloramphenicol can be employed with advantage as sole antibiotic. A standard procedure for the preparation and use of human amnion cells is described: The amnion is incubated with trypsin for 4 hours at 32° followed by 4 manual 'extractions'. Roux bottles are seeded with 50×10^6 cells suspended in 100 ml. growth medium consisting of Eagle's medium with lactalbumin hydrolysate 0·5%, supplemented with 10% human serum. Nystatin will keep yeast and mold contamination to a minimum. For use in the virus laboratory cells are removed from the Roux bottles by the combined action of trypsin and Versene, and transferred to smaller vessels as secondary cultures."

KELLER, K., BRASPENNINCKX, H. & KASDAN, M. **Detecting Viruses on Nonporous Surfaces by Use of the Cotton Swab Technique.** *Pub. Health Rep.* Wash. 1961, May, v. 76, No. 5, 453–8, 1 chart.

Swab-sampling methods are widely used to determine the degree and spread of bacterial contamination of eating utensils. In this investigation, similar methods were applied to the detection of virus contamination, with coliphage T1 and poliovirus type 1 as test organisms.

Crockery was deliberately contaminated with virus mixed with whole egg suspension, and allowed to dry. The dishes were then swabbed with non-absorbent cotton wool swabs dipped in phosphate buffer.

With coliphage T1 the method could detect an initial contamination of 16 particles in a 4 sq. inch test area, even after one hour air drying of the virus-egg suspension. With poliovirus type 1, tested in HeLa cell cultures, swabs taken within 5 minutes of contamination could detect as little as 12 TCD50 of virus inoculated over the test area. Poliovirus was, however, found to be sensitive to desiccation, and after contaminated plates had been dried for one hour, positive swab results were obtained only from plates initially inoculated with virus doses of the order of 600–6000 TCD50 per 4 sq. inches.

Derek Hobson

CAJAL, N., BURDUCEA, O., MATEESCU, S., MARINESCU, G., CEPLEANU, M. & COPELOVICI, Y. Evoluția unor viroze experimentale (gripă, poliomielită, Coxsackie, turbare, herpes) sub acțiunea radiofosforului (P^{32}) si a radioiodului (I^{131}). [**The Development of Experimental Virus Infections (Influenza, Poliomyelitis, Coxsackie, Rabies and Herpes) under the Influence of Radioactive Phosphorus (^{32}P) and Radioactive Iodine (^{131}I)**] *Studii si Cercetări Inframicrobiol.* 1961, v. 12, No. 1, 29–37. French summary.

The authors have investigated the course of a number of virus infections in mice weighing 14–18 gm. exposed to the action of internal beta-radiation in the form of disodium hydrogen phosphate containing ^{32}P, and to both beta- and gamma-radiation in the form of sodium iodide containing ^{131}I. The radioactive compounds were injected intraperitoneally in a single dose containing 25 μC. In mice infected intranasally with the T and PR8 strains of type A influenza virus, treatment with radioactive material given 90 minutes later caused an increase in mortality and a reduction in survival time in comparison with untreated control animals infected with the same dose of virus. In mice inoculated intracerebrally with 200 LD50 (for infant mice) of type A2 Coxsackie virus, treatment with the radioactive compounds caused the development of a fatal illness. The treatment had no effect in mice inoculated intracerebrally with type 1 (Mahoney) and type 3 (Saukett) poliomyelitis virus, but with type 2 (MEF 1) poliomyelitis virus the treatment caused an increase in mortality after intracerebral infection, and the development of illness with respective mortalities of 40–50% and 20–30% after intraperitoneal and subcutaneous infection. The treatment had an accelerating effect upon the infection in similar experiments with fixed rabies and herpes viruses. In conclusion, the authors put forward the hypothesis that an increase in natural radiation may cause an increase in morbidity due to virus infections. *D. J. Bauer*

SĂRĂTEANU, D., HUNG, T., DUMITRESCU, S. & IONESCU, N. I. Studiul comportării si modificărilor morfopatologice produse de tulpinile ornitozice pe membrana corioalantoidiană a embrionului de găină în dezvoltare. [**A Study of the Behaviour and Pathological Changes Produced by Strains of Ornithosis Virus on the Chorio-allantoic Membrane of the Developing Hen's Egg**] *Studii si Cercetări Inframicrobiol.* 1961, v. 12, No. 1, 83–94, 20 figs. on 10 pls. French summary.

The authors have studied the histopathology of infection with ornithosis virus after inoculation into the allantoic cavity of fertile eggs at the 9th day of incubation. Membranes examined 12 hours after infection showed thickening of the ectoderm due to proliferation of cells with red cell infiltration and occasional cell degeneration. The mesoderm was greatly thickened as a result of oedema and cell proliferation. After 24 hours the changes were more intense, and intracytoplasmic eosinophil inclusions surrounded by a halo could be seen in occasional ectodermal cells, and similar changes were seen in membranes examined at various times up to 120 hours after infection. *D. J. Bauer*

STRAUSS, J. & VECEREK, B. Pokusy o ovlivnění vnímavosti experimentálnich zvířat vůči viru ornitózy [**An Attempt to Influence the Sensitivity of Laboratory Animals to Ornithosis Virus**] *Českoslov. Epidemiol., Mikrobiol., Imunol.* Prague. 1961 v. 10, No. 2, 92–7, 4 graphs. [12 refs.] English summary.

In an attempt to increase sensitivity to ornithosis mice inoculated with the virus intracerebrally were

exposed to ultraviolet irradiation or treated with cortisone. In parallel titrations of a virus preparation carried out in normal mice and in irradiated animals LD50 titres ranging from 0·2 to 0·9 log unit higher were observed in the latter, and a titre 1·2 log unit higher was obtained in mice treated with 0·4 mgm. of cortisone. Some evidence of increased susceptibility to ornithosis infection after ultraviolet irradiation was obtained in experiments with pigeons. *D. J. Bauer*

CORAGGIO, F., ALTUCCI, P., PECORI, V., DE MARCO, G. & MARGHERITA, G. Studio della dinamica moltiplicativa del virus vaccinico e del virus EDP in culture di membrane corion-allantoidee *in vitro*. [**A Study of the Course of Multiplication of Vaccinia and EDP Viruses in Cultures of the Chorio-allantoic Membrane *in vitro***] *Riv. Istituto Sieroterap. Ital.* 1961, Mar.–Apr., v. 36, No. 2, 111–20, 2 graphs. [18 refs.] English summary.

The authors have studied the rate of multiplication of vaccinia virus and the EDP strain of ectromelia virus in roller tube cultures of fragments of chorio-allantoic membrane. After 2 hours of contact with the tissue the proportion of virus adsorbed amounted to 40% for vaccinia and 55% for ectromelia. New virus appeared in the membrane 6–12 hours after infection, and then increased in amount logarithmically after 48 hours with vaccinia, and for up to 4 days with ectromelia. At all times the titre of virus in the culture fluid was 1–2 log units lower than in the membrane. Histological examination of the infected tissues revealed the presence of diffuse cell hyperplasia after 36–48 hours, followed by degeneration and diffuse necrosis. Pocks were not formed. *D. J. Bauer*

NAGANO, Y.; KOJIMA, Y.; SUZUKI, T. Inhibition de l'infection vaccinale par un facteur liquide dans le tissu infecté par le virus homologue. II. Quelques propriétés biochimiques du facteur inhibiteur. [**Inhibition of Vaccinia Infection by a Factor from Tissue Infected by the Homologous Virus. II. Biochemical Properties**] [NAGANO, KOJIMA & SUZUKI]. *C.R. Soc. Biol.* 1960, v. 154, No. 11, 2166–8, 1 fig. III. Propriétés sérologiques du facteur inhibiteur. [**III. Serological Properties**] [NAGANO & KOJIMA]. *Ibid.*, 2172–5.

The authors remark the similarity between their factor and the interferon of ISAACS and LINDEMANN [this *Bulletin*, 1957, v. 32, 1203]. The factor was acid-stable and precipitable by ammonium sulphate. The activity was precipitated without loss by trichloracetic acid. It was not affected by ether, ribonuclease or deoxyribonuclease. It was destroyed by 0·2% trypsin in 24 hours, but not by 0·01% in 2 hours at 37°C. It was also destroyed by periodic acid. The authors conclude that their factor is proteinaceous, but cannot exclude a polysaccharide fraction.

The inhibiting factor was not related immunologically to any of the known antigenic components of vaccinia virus. By inoculating rabbits with factor and Freund adjuvant antibody was raised which was able to suppress the virus-inhibiting activity of the factor. *Colin Kaplan*

FALKE, D. & RICHTER, I. E. Mikrokinematographische Studien über die Entstehung von Riesenzellen durch Herpes-B-Virus in Zellkulturen. I. Mitteilung: Vorgänge an den Zellgrenzen und Granulabewegungen. [**Microcinematographic Studies of the Origin of Giant Cells due to Herpes B Virus in Tissue Culture. I. Occurrences at the Cell Boundaries and Movements of Granules**] *Arch. f.d. gesamte Virusforschung.* 1961, v. 11, No. 1, 73–85, 12 figs. II. Mitteilung: Morphologisches Verhalten und Bewegungen der Kerne. [**II. Morphological Behaviour and Movements of the Nuclei**] *Ibid.*, 86–99, 4 figs. [36 refs.]

I. By means of time-lapse photomicrography the authors have studied the events leading to the formation of giant cells in cultures of KB cells infected with herpes B virus. The cells were grown in a chamber sealed with coverslips and continuously irrigated with nutrient medium saturated with 95% oxygen and 5% carbon dioxide. The cells were photographed under a phase-contrast microscope at 2 frames a minute with intermittent illumination. Giant cells were first seen after 12 hours, and arose by confluence of cells with disappearance of intercellular boundaries, a process which lasted from 1 to 6 hours on each occasion. No multiplication of the nuclei occurred. Transient cell bridges were also observed, which could evidently afford an opportunity for infection of cells to occur without release of virus into the medium. Exchange of cell granules was seen to take place over the cell bridges.

II. By planimetric measurements of nuclear areas in serial photographs it was established that an increase in nuclear volume took place during giant cell formation, amounting to 40% 5 hours after fusion, and 100% after 14 hours. The contours of the nucleoli became obscure and masses appeared in the karyoplasm, although inclusion bodies were never observed. The nuclei were seen to wander over considerable distances; this may have been due to active movements, since there was no accompanying movement of cell granules. Rotation of the nuclei also occurred. *D. J. Bauer*

ROIZMAN, B. **Virus Infection of Cells in Mitosis. I. Observations on the Recruitment of Cells in Karyokinesis into Giant Cells induced by Herpes Simplex Virus and bearing on the Site of Virus Antigen Formation.** *Virology.* New York. 1961, Apr., v. 13, No. 4, 387–401, 1 diagram & 14 figs. (3 coloured) on 4 pls. [15 refs.]

Within 24 hours of infection with herpes simplex virus (MP strain) HEp-2 cells derived from a human epidermoid carcinoma form syncytia. The author has stained infected cultures with acridine orange and with herpes antibody labelled with fluorescein, and this paper, which includes some fine coloured photomicrographs, gives an account of some of the changes seen.

During the first 24 hours of infection syncytia contained numerous nuclei which, between 36 and 72 hours, gradually aggregated to form a large DNA mass within which the outlines of the nuclear membranes were still clear. Mitotic figures were seen as paired chromosomes arranged around the edge of 5–20% of syncytia. After 48 hours, cytoplasmic processes connecting syncytia to the cells adjacent to them were seen to be filled with herpes antigen and these cells also began to show nuclear changes. When colchicine was added to uninfected cells, 85% of the cells were arrested in metaphase by 24 hours. When colchicine and virus were added together, few syncytia formed and they were surrounded by cells in metaphase. No cytoplasmic processes connected syncytia with these cells. If colchicine was removed after 24 hours, unusual giant cells (macrokaryocytes) formed in which the nucleus contained clumps of chromosomes like those in metaphase. No cells in metaphase, anaphase or telophase stained specifically with fluorescent antibody. Antigen first gathered in the perinuclear space and only entered the cytoplasm and cytoplasmic projections 24–48 hours after infection. These results support electron microscope observations on the site of herpes formation made by MORGAN and co-workers [this *Bulletin*, 1960, v. 35, 311]. In macrokaryocytes antigen formed round the rim of the mitotic figures.

The author concludes that cells in the various phases of mitosis react differently to virus and to nearby infected cells. Cells in prophase or interphase most commonly formed syncytia and mitotic figures were observed, it seems, when nuclear division had already begun before the cells fused. Colchicine, apart from stopping mitosis in metaphase, acted separately to prolong the phase in which cells resisted forming syncytia, so that after its removal cells formed macrokaryocytes although they were still in metaphase. This resistant phase appears to be independent of the behaviour of the chromosomes. *Janice Taverne*

NOJIMA, T. **A Mutant of Herpes Simplex Virus possessing an Attenuated Pathogenicity for Baby Mice obtained by Egg Passage of a Newly Isolated Strain.** *Japanese J. Med. Sci. & Biol.* 1960, Dec., v. 13, Nos. 4, 5 & 6, 179–89, 1 fig. [27 refs.]

The SK strain of herpes simplex virus, isolated from a patient with eczema herpeticum and subjected to 6 serial passages in the chick chorio-allantoic membrane, grew to the same titre in eggs and in suckling mice, whether injected intracerebrally or intraperitoneally. Between the 14th and 25th passages the strain decreased in pathogenicity for suckling mice, the change being most marked when the mice were injected intraperitoneally. (Where formerly there had been less than a log difference in titres obtained from pock counts and mouse titrations, the latter now gave titration results 4 logs lower.) By passage 62 its virulence for the rabbit cornea had also diminished, but only slightly, and there was no correlation with the loss of virulence for the baby mouse. When virus from the 63rd egg passage was passed serially in suckling mouse brain 4 times it reverted to its original level of virulence. In contrast, the HF strain of herpes maintained its pathogenicity fo suckling mice after more than 400 egg passages.

The author believes that a mutation occurred in th SK strain between passages 14 and 25, and that wit further passage the mutant gradually outgrew the paren type. However, it had not, it seems, completely elimi nated the parent by passage 63 since mouse pathogeni city was restored by selection of the parent by passag in mouse brain. *Janice Taverne*

DANIELESCU, G., BOERU, V., ADERCA, I. & IANCO NESCU, M. Modificările metabolismului glucidic în membranele corioalantoide *in vitro*, sub influenţa interferonului. [**Modifications in the Glucos Metabolism of the Chorio-allantoic Membrane *in vitro* under the Influence of Interferon**] *Studi si Cercetări Inframicrobiol.* 1961, v. 12, No. 1 109–15. [11 refs.] French summary.

Pieces of chorio-allantoic membrane weighing 18–2(mgm. were incubated in a preparation of interferon in Earle solution derived from the P strain of type A influenza virus; determinations were made of the glucos content of the medium before and after incubation, an also of control preparations consisting of pieces o membrane in Earle solution. The latter reduced th glucose content of the medium from the initial value o 100 mgm. per 100 cc. to 60·7 mgm. [in Table 3; 66·7 i Table 1] over a period of 24 hours, whereas in the pre sence of interferon glucose utilization was reduced, th level falling to only 79·5 mgm. per 100 cc. over the same period. The glucose levels were closely similar during the first 5 hours of incubation, and the inhibiting effec of interferon became apparent from 7 hours onwards *D. J. Bauer*

ALTUCCI, P., CORAGGIO, F., MARGHERITA, G. & GALEOTA, C. Analisi di alcuni aspetti quantitativ della dinamica moltiplicativa del virus influenzale A-PR8 in Culture di membrane corion-allantoide *in vitro*. [**The Multiplication of Influenza Virus A-PR8 in Chorio-allantoic Tissue Culture**] *Riv Istituto Sieroterap. Ital.* 1961, Mar.–Apr., v. 36 No. 2, 90–110, 6 graphs. [50 refs.] Englis summary.

A study has been made of the course of multiplicatio of the PR8 strain of influenza virus in roller tube cul tures of pieces of chorio-allantoic membrane. Th amount of virus adsorbed rose from 50% with an infec tive inoculum of 1000 EID50 per cell, to nearly 98% with a dose of less than 0·1 EID50 per cell. The loga rithm of the virus content of the membrane as deter mined by titration in eggs fell from an initial value o 3·2 EID50 per 0·025 gm. to a minimum of 2·5 after hours, thereafter rising to a maximum of 6·5 after 2 hours. Infection of cultures of membrane was a some what less sensitive method of detecting influenza viru than infection of intact eggs, but was considerably mor sensitive than any other *in vitro* method. *D. J. Bauer*

PORTOCALĂ, R.; BOERU, V.; SAMUEL, I.; ANDREESCU, M. Reproducerea virusului gripal cu ajutorul acidului ribonucleic virotic. IV. Cercetarea ribonucleazei în ouăle embrionate de diferite vîrste [**The Reproduction of Influenza Virus by means of Virus Ribonucleic Acid. IV. Investigation of Ribonuclease in Fertile Eggs of Different Ages**] [PORTOCALĂ, BOERU & SAMUEL]. *Studii si Cercetări Inframicrobiol.* 1961, v. 12, No. 1, 71–5. French summary (5 lines). V. Influența calității fenolului asupra activității acidului ribonucleic [**V. The Influence of the Purity of the Phenol upon the Activity of the Ribonucleic Acid**] [PORTOCALĂ & ANDREESCU]. *Ibid.*, 77–81. French summary.

IV. In a continuation of previous work on the infectivity of ribonucleic acid isolated from influenza virus the ribonuclease content of the uninfected chorio-allantoic membrane and allantoic fluid has been determined at various stages of incubation. The activity of the enzyme, expressed as μgm. of phosphorus per 100 mgm. of membrane or 1 cc. of allantoic fluid liberated from ribonucleic acid under the particular experimental conditions, rose in the membrane from 33 on the 8th day to 104 on the 14th day of incubation, and in the allantoic fluid from 8 to 28.

V. The authors have previously observed that some samples of ribonucleic acid (RNA) isolated from influenza virus lack infectivity, and in the present work 4 commercial samples of phenol (employed in extraction of RNA) obtained from the Soviet Union, Czechoslovakia, Germany and the United States have been analysed to determine the nature and concentration of the impurities present. The results are presented in a table. Among other metal ions lead was found in amounts up to 0·00093 gm.%, a concentration which was considered to be sufficient to account for the lack of infectivity of the nucleic acid.

[See this *Bulletin*, 1960, v. 36, 312.] *D. J. Bauer*

MOGABGAB, W. J., HOLMES, Barbara & PELON, W. **Influenza A and B Viruses in Monkey Kidney Cultures. I. Properties of the Viruses.** *J. Infect. Dis.* 1961, May–June, v. 108, No. 3, 315–23, 3 figs.

Influenza A1, A2 and B strains previously passaged allantoically in eggs or in human embryonic tissue culture were studied in primary rhesus monkey tissue (MK) cultures maintained at 35°C. in serum-free medium 199 held at *p*H 7·0–7·4.

Growth rates and virus yield did not become reproducible until the 3rd or 4th passage in MK cultures, suggesting an adaptive phase even with virus strains already adapted to human embryonic tissues. With all strains, the growth rates remained slower than in human tissue or in eggs, and incubation for 7 days was advisable in calculating infectivity titres or neutralizing antibody titres.

Certain virus properties of egg-grown viruses became modified after adaptation to MK tissue culture; the MK tissue-culture line became more reactive with monkey erythrocytes and less sensitive to non-specific haemagglutination-inhibitors in monkey serum though sensitivity to serum-inhibitors in other species increased. Virus infectivity of MK-grown virus was more stable over the temperature range 35–60°C. than egg-lines of virus, but became sensitive to the neutralizing activity of a component of normal rabbit serum sensitive to receptor destroying enzyme. *Derek Hobson*

BARRY, R. D. **Equilibrium Sedimentation of Influenza Virus in Caesium Chloride Density Gradients.** *Australian J. Exper. Biol. & Med. Sci.* 1960, Dec., v. 38, Pt. 6, 499–507, 8 figs. [16 refs.]

Influenza A virus MEL and PR8 were mixed with caesium chloride solutions with an initial density of 1·24 gm. per ml. and centrifuged for 40 hours at 33,000 r.p.m. in the Spinco swinging bucket head. Well defined peaks of haemagglutinin activity were found in the middle fractions recovered from these tubes. 20% of the haemagglutinin of PR8 virus appeared to be less dense than the main component and when first and second passage "incomplete" virus preparations were made there was a larger proportion of less dense haemagglutinin but without any clearly defined secondary peaks. Virus enzyme measurements were made on fractions from the standard PR8 virus and electron microscopic particle counts were made on the fractions from standard MEL virus. In both cases the quantities found were correlated closely with the titres of haemagglutinin, and this suggested that the virus particle could not be separated into different species by this density gradient method. It was impossible to measure infectivity on the fractions as the high concentrations of caesium or possibly some associated impurity were able to inactivate the virus. *D. A. J. Tyrrell*

KATO, N. & OKADA, A. **The Relation of the Toxic Agent to the Subunits of Influenza Virus Particles.** *Brit. J. Exper. Path.* 1961, June, v. 42, No. 3, 253–65, 2 figs. on pl. [17 refs.]

Influenza A virus was purified by adsorption and elution on red cells and on aluminium phosphate columns. This virus was treated with sonic vibrations for periods up to 2 hours. The haemagglutinin titre was increased, infectivity greatly reduced, and S antigen was released. The haemagglutinin (HA) particles and S antigen were separated and examined under the electron microscope and the sedimentation coefficient ($S_{20,w}$) of the HA particles at infinite dilution was 74 Svedberg units, while that of the original preparation was 752. Virus toxicity was retained by virus preparations disrupted in this way and it was associated mainly with the HA fraction. Treatment with heat, ultraviolet irradiation and sodium ethylmercurithiosalicylate destroyed toxicity without affecting the HA titre. The pathological changes induced in animals given the toxic HA preparations were almost indistinguishable from those induced by injection of untreated toxic virus particles. The authors conclude that "the majority of the toxic agent is located near the surface of the virus particle with an intimate relation to the haemagglutinin"

and discuss the relationship between their findings and those of others in this field. [This paper is well worth reading in the original.] *D. A. J. Tyrrell*

HÁNA, L. & STYK, B. **Lipoids as Inhibitors of Haemagglutination-Inhibiting Activity against "Avid" Strains of A2 Influenza.** *Acta Virologica.* Prague. 1961, May, v. 5, No. 3, 190–92, 1 fig. on pl.

The inhibitory activity of guineapig serum against "avid" influenza A2 virus is increased by the delipidization of the serum by McFarlane's method. Electrophoretic examination of the serum shows that the alpha globulin lipoprotein band, which carries the inhibitory activity in untreated and treated sera, stains more strongly for glycoproteins after delipidization. The authors conclude that the greater inhibitory activity and the increased staining of delipidized sera are due to "unmasking" of groups previously combined with lipid. [See also this *Bulletin*, 1961, v. 36, 499.]

D. A. J. Tyrrell

ALBRECHT, P., KRIZANOVÁ, O. & SZÁNTÓ, J. **Location of Influenza Virus Haemagglutination Inhibitor demonstrated in Chick Embryo Cells by Fluorescent Antibodies.** *Acta Virologica.* Prague. 1961, July, v. 5, No. 4, 232–5, 6 figs. on 3 pls. [15 refs.]

"The distribution of the virus haemagglutination inhibitor in tissues of 11 to 12 days old chick embryos was studied by fluorescent antibodies. The inhibitor was demonstrated in epithelial cells lining the allantoic cavity and in some internal organs (oesophagus, stomach, kidneys, oropharynx).

"The location of the IVH [inhibitor of virus haemagglutination] in the chorioallantoic membrane, oesophagus and stomach could be confirmed by positive haemagglutination inhibition activity of extracts from these tissues.

"Considering the location of the IVH in the chorioallantoic membrane and tissue cultures it can be assumed that the IVH is produced throughout the cytoplasm of the epithelial cells and is periodically eliminated onto the cell surface."

BORECKÝ, KOCISKOVÁ, D. & HÁNA, L. **An Attempt to affect the Level of Serum Inhibitors of Myxoviruses *in vivo*. I. Effect of Ethionine Administration on the Level of Inhibitors in Guinea Pig Sera.** *Acta Virologica.* Prague. 1961, July, v. 5, No. 4, 236–44, 4 text figs. & 1 fig. on 2 pls. [14 refs.]

"The repeated intraperitoneal administration of a 2·5% solution of d-l ethionine to female guinea pigs of different weights caused a progressive decrease in the level of serum inhibitors showing virus neutralizing and antihaemagglutinating activity against Newcastle disease and A1 type (strain A1/Čs 1/51) influenza viruses. No change in the level of inhibitors against avid A2/Singapore/57 influenza virus was observed. The significance of these findings is discussed."

STYK, B. & HÁNA, L. **The Effect of Inhibitor-Destroying Substances on the Cofactor and Specific Antihaemagglutinins against A2 Influenza Virus.** [Correspondence.] *Acta Virologica.* Prague. 1961 May, v. 5, No. 3, 194.

The authors have described a heat-labile co-factor which is necessary for the manifestation of haemagglutination-inhibiting activity by early immune mouse antibody against "non-avid" influenza A2 [this *Bulletin*, 1960, v. 35, 89]. The co-factor activity was destroyed by M/50 KIO_4 and not by 1% trypsin, whereas beta inhibitor was not destroyed by KIO_4 but was by trypsins; both were destroyed by CO_2 treatment. M/90 KIO_4 apparently destroyed antibody. *D. A. J. Tyrrell*

CHANG, Shueh-Shen & CHEN, Chao-Yuan. **Inhibitor Spectra and Effects of Various Treatments on the Inhibitors of Influenza Virus Hemagglutination.** *J. Formosan Med. Ass.* 1960, Oct. 28, v. 59, No. 10, 1110–22. [19 refs.]

Haemagglutination inhibition titrations with 12 viruses (influenza A, A1, A2, swine, B, C and Sendai) were performed against sera of 8 animal species and extracts of various chick and mouse organs. The results are presented *in extenso*. The effects are reported of treatment by standard methods with CO_2, KIO_4, trypsin and RDE (receptor-destroying enzyme) or certain non-specific inhibitors, mainly sera, and also or immune sera. Many results reported have been described elsewhere; the authors find CO_2 "removed some of the Francis- and Chu-inhibitors" (α- and β-inhibitors). KIO_4 was most effective against Francis and Asian influenza inhibitors but affected the Chu inhibitors but little. It apparently destroyed antibody. The Asian virus inhibitors were not destroyed by RDE, but those in rabbit and human sera (but not in guineapig and horse sera) were destroyed by trypsin if the sera were heated before treatment. *D. A. J. Tyrrell*

LINK, F., BLASKOVIC, D. & RAUS, J. **Studies on the Effect of Antiviral Substances on Experimental Virus Infections. II. Dose Response Relationship between Concentration and Antiviral Effect of Urethane in Mice.** *Acta Virologica.* Prague 1961, July, v. 5, No. 4, 245–9, 4 figs.

"Urethane given in 2·5%, 5% and 25% concentrations to mice infected with the mouse-adapted Em strain of type A1 influenza virus lowered the mortality rate of the animals. The mortalities were reduced as in the mice had been infected with virus concentrations 0·5, 0·75 and 1·4 $\log_{10}$ respectively, below that of the control mice. There was a linear relationship between the dose of urethane and the apparent reduction of titre of the virus inoculated."

[For Part I see this *Bulletin*, 1961, v. 36, 814.]

DICK, E. C.; MOGABGAB, W. J.; HOLMES, Barbara. **Characteristics of Para-Influenza 1 (HA-2) Virus. I. Incidence of Infection and Clinical Features in Adults.** [DICK, MOGABGAB & HOLMES]. *Amer. J. Hyg.* 1961, May, v. 73, No. 3, 263–72, 1 fig. [15 refs.] **II. Hemagglutination, Hemagglutination-Inhibition and Neutralization** [DICK & MOGABGAB]. *Ibid.*, 273–81, 2 figs. [21 refs.]

The first paper reports the investigation of adult respiratory infections in Louisiana and Mississippi. Garglings from patients were inoculated into embryonated eggs, and HeLa and rhesus monkey kidney tissue cultures.

From 749 specimens, 16 strains of parainfluenza type 1 virus were recovered on monkey kidney but none on HeLa or in eggs. Cytopathic changes were minimal but all strains were readily detected on first passage by haemadsorption or haemagglutination.

Two of the strains isolated were compared in haemagglutination-inhibition tests with the prototype strains of parainfluenza 1. The New Orleans strains, Copenhagen 222 and HA2 appeared identical, and all four showed a similar distant relationship to Sendai virus.

Parainfluenza 1 infection, as judged by virus isolation and serological response, accounted for 2·6% of all adult respiratory disease, the highest incidence being autumnal. The clinical disease was mild, with sore throat and nasal congestion as the main features.

Almost 50% of acute phase sera from all respiratory infections showed resting antibody titres greater than 1 in 16, whereas 11 people with proved parainfluenza 1 infections had little or no detectable antibody in the acute illness serum sample.

The second paper reports some factors involved in haemagglutination-inhibition and virus neutralization methods with the Copenhagen 222 strain of parainfluenza 1.

Human group O erythrocytes gave the most reproducible results with human sera ; preincubation of virus and serum for 30 minutes before the addition of red cells was necessary, and the sensitivity of haemagglutination and haemagglutination-inhibition was greater at room temperature than at 4°C. Treatment of human sera with cholera filtrate was essential to remove nonspecific inhibitors of haemagglutination.

In a rather small series of paired sera haemagglutination-inhibition results were comparable with those obtained by complement fixation or neutralization methods.

In neutralization experiments, the virus inactivation curves obtained showed a steep slope similar to that of other myxoviruses, and the degree of neutralization was much greater in serum-virus mixtures held at 35°C. for 1 hour than at room temperature or 4°C. for longer periods. *Derek Hobson*

SOKOL, F., BLASKOVIC, D. & KRIZANOVÁ, O. **Subunits of Myxoviruses. II. Properties of Haemagglutinins of Newcastle Disease, Para-Influenza 1 and Mumps Viruses.** *Acta Virologica.* Prague. 1961, May, v. 5, No. 3, 153–9, 1 fig. on pl. [10 refs.]

The viruses were disrupted by ether treatment as described earlier [this *Bulletin*, 1961, v. 34, 813] and the subunit suspensions were adsorbed on to erythrocytes and haemagglutinin (HA) was eluted from them. More HA activity was recovered than was present originally and there was electron microscope evidence that the larger units were replaced by smaller spherical units 30 m_μ in diameter. The HA thus prepared from NDV was precipitated by distilled water ; HA from all viruses were not sedimented by 36,000 g for 45 minutes, although the virus haemagglutinin was ; haemolytic activity was not observed but HA of parainfluenza and NDV had high neuraminidase activity as judged by the release of sialic acid. The results are discussed and it is suggested that those particles might be compared with the " small " haemagglutinin extracted by GRANOFF [*ibid.*, 1956, v. 31, 592] from cells infected with NDV. *D. A. J. Tyrrell*

FINKELSTEIN, R. A. **Alteration of Susceptibility of Embryonated Eggs to Newcastle Disease Virus by *Escherichia coli* and Endotoxin.** *Proc. Soc. Exper. Biol. & Med.* 1961, Mar., v. 106, No. 3, 481–4, 2 figs. [13 refs.]

Chick embryos were inoculated allantoically with *E. coli* or endotoxin prepared from that organism, and then with a small dose of NDV (Newcastle Disease virus). The death of embryos was recorded. If *E. coli* was administered 12–72 hours before the virus the mortality was less than in embryos inoculated with virus alone. If bacteria were inoculated later than this then the mortality was greater than in controls. The inhibitory effect was shown when endotoxin was administered instead of bacteria, and also slight enhancement of mortality. *D. A. J. Tyrrell*

POWELL, H. M., WALCHER, D. N. & MAST, C. **Inhibition of Cytopathic Action of Salisbury Virus by Antiviral Agent 1758.** *Proc. Soc. Exper. Biol. & Med.* 1961, May, v. 107, No. 1, 55–7.

The virus HGP obtained from a case of the common cold syndrome occurring in Salisbury was propagated in monkey kidney tissue cultures in which it produces a cytopathic effect. The antiviral agent 1758 (also called Statolon) was obtained from cultures of *Penicillium* and used in the form of a partially concentrated broth. The agent was added to tissue culture tubes which were then incubated for 24 hours and infected with HGP virus. No cytopathic effect developed although virus could be demonstrated to be present in the tubes by subculture into untreated tissue cultures. The effect was abolished by boiling 1758 for 5 minutes. The agent was active at dilutions of up to 1/5 or 1/10 depending on the dose of virus given. *D. A. J. Tyrrell*

Derevici, A. & Istrati, M. Cercetări comparative în diagnosticul adenovirozelor prin reactia de fixare a complementului si a precipitării în gel de agar. **[Comparative Investigations in the Diagnosis of Adenovirus Infections by the Complement-Fixation Test and Precipitation in Agar Gel]** *Studii si Cercetări Inframicrobiol.* 1961, v. 12, No. 1, 63–70, 6 figs. [14 refs.] French summary.

The results obtained by the complement-fixation test and gel diffusion precipitation with type 4 adenovirus antigen in the diagnosis of adenovirus infection have been compared in the examination of 479 sera from healthy persons and from patients with acute respiratory infections. Positive results with the gel diffusion test were generally associated with high complement-fixation titres. Thus, only 19 of 243 sera lacking complement-fixing antibody gave a line in the gel diffusion test, compared with 22 of 38 sera with a complement-fixation titre of 64, and 13 of 14 with a titre of 128.

D. J. Bauer

J. Amer. Med. Ass. 1961, May 27, v. 176, No. 8, 647–67, 4 figs. [Numerous refs.] **Respiratory Syncytial Virus.**

R. M. Chanock, H. W. Kim, A. J. Vargosko, Ann Deleva, K. M. Johnson, Christine Cumming and R. H. Parrot, in a study of acute lower-tract respiratory disease at the Children's Hospital, Washington (pp. 647–653), isolated only 4 strains of respiratory syncytial (RS) virus between October 1957 and February 1960. However, Beem *et al.* [this *Bulletin*, 1961, v. 36, 162] observed that isolation of the virus was facilitated if specimens were inoculated into tissue cultures without prior freezing and thawing; using this technique Chanock *et al.* isolated 56 strains of RS virus from 346 patients with respiratory disease and 4 strains from 272 control subjects fom March to July 1960 inclusive. Throat swabs were collected in Hanks solution with 0·5% gelatin and the isolations were made in HEp-2 tissue cultures, maintained in Eagle's basal medium with 5% inactivated chicken serum, and incubated at 36°C. The characteristic syncytial cytopathic effect was not recognized until at least 6 days after inoculation and the maximum time was 22 days; an isolate was classified as RS if it produced this effect and completely fixed complement with 4 units of a guinea-pig antiserum. The virus was recovered from 42% of infants with bronchiolitis, 24% of children with pneumonia and 12% of children with other minor respiratory infections, usually febrile; virus was recovered most frequently (57%) from infants less than 7 months old with bronchiolitis or pneumonia but with minor respiratory illness the proportion of virus-positive individuals remained about the same until the fifth year of life. Of 34 patients with lower respiratory tract illness from whom virus was isolated, 21 developed a rise in antibody during convalescence; 16 patients over 4 months of age developed neutralizing antibody but only 5 of 18 younger infants had such a response. A similar trend was evident for complement-fixing (CF) antibody. The sensitivity of the CF test was increased 47% by using 8 units instead of 4 units of antigen, but even so it detected only half the virus infections that could be recognized by virus isolation.

Association of the RS virus with disease and knowledge of the CF antibody response in hospital patients from whom virus was isolated made it possible for R. H. Parrot, A. J. Vargosko, H. W. Kim, Christine Cumming, H. Turner, R. J. Huebner and R. M. Chanock (pp. 653–657) to interpret the CF antibody data acquired since October, 1957. During the 3-year study there was serological evidence of infection with RS virus in 11% of 1,038 children with respiratory disease; this was 5·5 times more than in the controls. However, CF antibody titres of most of the sera were determined with 4 units of antigen (the less sensitive method, as noted above). After adjusting the results for the known CF insensitivity, it was estimated that 21% of the patients were infected with RS virus; 36% of all bronchiolitis patients and 21% of all broncho-pneumonia patients were estimated to have been infected with RS virus and most often infants rather than older children.

Chanock *et al.* [see above] noted that virus isolations were made over periods of 3 to 5 months and not throughout the year; in an addendum to the first paper the authors report a further period of virus isolations from November 1960 to February 1961 inclusive. These circumscribed outbreaks of infection were supported by the serological data, for there were sharp rises in the CF antibody responses in November 1958 to February 1959 and again in March–July 1960, the latter corresponding with the first large number of virus isolations. Furthermore, the peaks of occurrence of bronchiolitis coincided with periods of virus prevalence and thus confirmed the evidence that RS infection is a prominent cause of bronchiolitis in infants.

H. Kravetz, V. Knight, R. M. Chanock, J. A. Morris, K. M. Johnson, D. Rifkind and J. P. Utz (pp. 657–663) studied the effect of RS virus inoculation in adult male volunteers (21–35 years of age) from several federal correctional institutions. After routine examination, the volunteers were isolated, usually 3 per room, for 3–6 days before and for about 10 days after inoculation. The inoculum contained 160–640 TCID50 of second monkey-kidney passage RS virus given as 1·0 ml. sprayed into the nose and throat and 0·5 ml. instilled into each nostril. Although a simian vacuolating virus (SV_{40}) was also present, evidence is presented which suggests that this did not cause illness nor influence the response to the RS virus. The latter was given on 5 different occasions to a total of 41 volunteers, and 20 developed a cold-like illness; RS virus was recovered from 17 of them (85%) and 16 showed a 4-fold CF antibody rise. Although 21 volunteers did not become ill, RS virus was recovered from 13 of them (62%) of whom 2 had a 4-fold CF antibody response and another developed serological evidence of infection. There was no cultural or serological evidence of infection in 18 subjects given control fluid. The incubation period lasted 3–7 days (average 4·9) and the illness lasted 2–11 days (average 5·5). Virus isolations were usually positive 24 hours before the onset of illness and continued to be so for the next 4–5 days.

The illness was characterized mainly by nasal discharge and stuffiness without fever.

K. M. Johnson, R. M. Chanock, D. Rifkind, H. M. Kravetz and V. Knight (pp. 663–667) re-emphasized the association between virus challenge and subsequent illness and showed further that all volunteers possessed neutralizing antibody before infection. With one exception the titres ranged from 1 in 16 to 1 in 256. However, neither infection, as judged by virus recovery, nor clinical illness, was influenced by the level of pre-existing antibody. Virus was recovered from 19 of 25 subjects with low levels (1/32 or less) and from 11 of 16 with high levels (1/64 or more) of neutralizing antibody. Clinical illness was also observed with equal frequency in both groups. The presence of pre-existing antibody suggested previous infection and the mild afebrile nature of the illnesses fitted in with this thesis of reinfection. The fact that individuals with high levels of antibody were infected suggested that prevention of RS infection in adults by vaccination might prove difficult. *D. Taylor-Robinson*

Mares, I., Drevo, M. & Slonim, D. **A Study on the Prevention of Poliomyelitis Virus Adsorption on Seitz Filter Discs.** *Acta Virologica.* Prague. 1961, July, v. 5, No. 4, 220–27, 3 figs.

Filtration of poliovirus through untreated Seitz pads results in loss of most of the virus by adsorption. Experiments to devise a way of preventing this loss in the preparation of living and inactivated poliovaccine are described. The best method was found to be washing the filters with 0·03% gelatin in Earle's solution at a rate of 4–5 ml. per cm.^{2} of pad. 60–120 ml. per cm.^{2} of virus fluid may satisfactorily be filtered through the pad, 60 ml. being the minimum quantity for good results. This treatment did not affect the effectiveness of the filter for sterilization. *A. J. Beale*

Peizer, Lenore R., Mandel, B. & Weissman, Dora. **An Improved Method for Rapid Laboratory Diagnosis of Poliomyelitis.** *Proc. Soc. Exper. Biol. & Med.* 1961, Apr., v. 106, No. 4, 772–6, 1 fig.

The presence of non-specific cytotoxins in virus-containing faecal extracts frequently hampers isolation procedures and direct serological identification of virus in tissue cultures.

This paper reports methods for overcoming these disadvantages; 20% suspensions of faeces in Hanks solution without bicarbonate were centrifuged at 2,000 r.p.m. for 10 minutes, the supernatant recentrifuged at 8,000 r.p.m. for 20 minutes in the Spinco model L centrifuge, and this second supernatant centrifuged at 38,000 r.p.m. for 2 hours in the presence of 0·1% gelatin. The deposit was resuspended in 2 ml. of tissue culture medium, and was used for all subsequent isolation and identification experiments.

Portions of the partially purified concentrate containing virus were incubated with poliovirus types 1, 2 and 3 antisera, singly and pooled, or saline for 45 minutes at room temperature before inoculation on to monkey kidney or HeLa cell cultures.

The efficiency of segregation of virus from faecal matter was estimated by performing virus plaque counts at each stage of the process. In fact, considerable quantities of virus were being discarded in the final supernatant, but the virus concentration in the deposit was at least 5 times greater.

In 176 stool specimens, 58 were found positive by conventional methods, with 18 non-specific cytotoxic reactions; by the purification method, 60 were found positive with no cytotoxic reactions, and 80% of these viruses were typed within 5 days of receipt.

[This method may be of use to the routine diagnostic laboratory with ample time and staff for lengthy preparation of unknown specimens, and in possession of a Spinco centrifuge.] *Derek Hobson*

Crowther, D. & Melnick, J. L. **The Incorporation of Neutral Red and Acridine Orange into Developing Poliovirus Particles making them Photosensitive.** *Virology.* New York. 1961, May, v. 14, No. 1, 11–21, 5 figs. [20 refs.]

Monkey kidney tissue culture monolayers are known to become photosensitive to white light in the presence of neutral red (NR). The cell-sheets may degenerate spontaneously, and polioviruses titrated under such conditions are known to yield plaques reduced in size and in number.

In the present experiments, when the NR concentration was reduced to 1 in 60,000 and light exposure limited to 15 minutes no obvious cell damage occurred but there was still a significant reduction in the efficiency of plating and plaque size of virulent and avirulent strains of poliovirus types 1, 2 and 3. The degree of reduction was similar (approx 50%) for both virulent and avirulent strains.

The effect of light on developing plaques was studied; exposure to light for 15 minutes after 5 days' incubation in the dark was still effective in slowing the subsequent increase in plaque size with both Mahoney and LSc strains.

In single-cycle growth curves of poliovirus in varying concentrations of NR, a 100,000-fold fall in virus yield occurred on increasing the NR concentration from 1 in 300,000 to 1 in 30,000, with a constant short period of light exposure.

When light was applied at various times in the poliovirus growth-cycle it could be shown that the inhibitory effect was exerted on fully-formed virus as well as upon early developmental stages.

Mature virus grown in the absence of NR was not sensitive to later applications of light plus NR, whereas virus grown in the dark in cells containing NR was subsequently photosensitive. To some extent, the degree of photosensitivity was proportional to the concentration of dye, but high concentrations of NR alone inactivated virus even in the absence of light.

When poliovirus-infected tissue-culture monolayers were stained with acridine orange or fluorescent antibody it was shown that synthesis of RNA was not significantly inhibited by NR despite the marked effect

on infectivity titres of virus. Other photodynamically-active dyes were compared with NR; not all were active, but acridine orange and proflavine (structurally very similar to neutral red) gave similar virus-inhibitory effects. Proflavine has been used widely in virus maturation studies with inhibitory effects varying from strain to strain and from laboratory to laboratory. It seems possible that the role of light-exposure in such investigations will need reconsideration.

Derek Hobson

FURNESS, G. **The Effect of Environment on the Replication of Poliovirus in Monkey Kidney Cells.** *J. General Microbiol.* 1961, July, v. 25, No. 3, 421–8, 3 figs. [11 refs.]

Poliovirus particles and numbers of infected cells were estimated by a modification of the plaque technique in which virus or infected cell suspension was added to 0·6% agar in 1 ml. and poured over a monolayer of cells in a 6 cm. Petri dish. It was found that 70% of the adsorbed virus went into eclipse in 1 hour at 25°C. or 37°C. At 37°C. the eclipse period lasted 3–4 hours and then the yield was about 700–1400 plaque forming units per cell. At 25°C. virus maturation did not occur, but on raising to 37°C. virus synthesis started anyway after up to 10 hours at 25°C. The length of eclipse phase and yield varied with *p*H; at *p*H 6·0–6·5 the eclipse phase was increased, and at over 8·0 the yield declined markedly.

A. J. Beale

KRITCHEVSKY, D., CARP, R. I. & KOPROWSKI, H. **Effects of Heavy Water on Poliovirus Multiplication: Results and Speculations on Mechanism.** *Nature.* 1961, July 15, v. 191, 250–51. [19 refs.]

It has previously been shown that cells in D_2O (deuterium oxide) produce more poliovirus that the same cells in watery media [CARP *et al.*, this *Bulletin*, 1961, v. 36, 203]. It has also been found that in media containing D_2O virus production at lower temperatures (33°C.) is inhibited and at normal (37°C.) and higher temperatures (40°C.) is increased (LWOFF and LWOFF, *C.R. Acad. Sci.*, 1960, v. 251, 3131; 1961, v. 252, 223). The authors have investigated the mutagenic effect of D_2O on CHAT poliovirus. It was found that virus exposed for 18 hours at 40°C. and then at 37°C. for the remainder of 4 days produced more and larger plaques than virus in the control preparation or virus kept in D_2O at 37°C. only. D_2O increases cell size, and cell size increased by X-rays has been found to increase the virus yield per cell (LEVINE and KRITCHEVSKY, *Exper. Cell Res.*, 1958, v. 15, 422), but the effect of D_2O on virus yield appears to be greater than that of X-rays.

A. J. Beale

KUNIN, C. M. & JORDAN, W. S., Jr. ***In vitro* Adsorption of Poliovirus by Noncultured Tissues. Effect of Species, Age and Malignancy.** *Amer. J. Hyg.* 1961, May, v. 73, No. 3, 245–57, 3 figs. [35 refs.]

Poliovirus grows in cells tissue-cultured from many organs of primates yet in the intact animal it is presumed to multiply only in the alimentary canal and CNS. The authors, influenced by the work of MCLAREN *et al.* [this *Bulletin*, 1959, v. 34, 953], sought virus receptors in tissue minces from monkeys, man and mice. They found that adsorption of virus occurred to a variety of monkey tissues (heart, muscle, gut, brain, lung, liver, testis, spleen) and that adsorption was prevented by heating the tissues or by treating them with ether or trypsin as described by McLaren *et al.* for the destruction of poliovirus cell receptors. Experiments with human tissues were hampered by the presence of a heat-stable virus inhibitor (presumably antibody) in the homogenates. No adsorption occurred to mouse tissues or to full term amnion, but adsorption did occur to some human malignant tumour homogenates. The authors, in discussion, think that these results make the problem of tissue tropisms in poliomyelitis more difficult to understand.

A. J. Beale

ORSI, E. V., MESSORE, G., LEVINE, S. & ROCA-GARCIA, M. **Serum Inhibition of Cytopathic Effect and Virus Release of Type 3 Poliovirus Vaccine in Primary Monkey-Kidney (MK) Cultures.** *Amer. J. Hyg.* 1961, May, v. 73, No. 3, 282–93, 4 pls. & 3 figs. [25 refs.]

A delay in the production of cytopathic effects in monkey kidney cells by the Lederle type 3 attenuated poliovirus strain occurred in the presence of chick serum. This effect was not noted in serum-free media, or with other strains of poliovirus, attenuated or virulent. The effect was found to be due to a delay in release of virus although the titre of cell-associated virus was undiminished.

A. J. Beale

GHENDON, Y. Z., KHESIN, Y. E., ROZINA, E. E. & MARCHENKO, A. T. **Investigations into the Viraemia caused by Sabin's Attenuated Poliovirus Strains.** *Acta Virologica.* Prague. 1961, July, v. 5, No. 4, 201–9. [11 refs.]

Viraemia in monkeys following the intramuscular inoculation of Sabin's vaccine strains of poliovirus, their progeny from vaccinated children and after passage in the laboratory (in tissue culture or monkeys), was investigated. It was found that viraemia did not occur with the type 1 or 3 strains but that 1 out of 8 monkeys developed transient viraemia after type 2. All 3 types together caused no viraemia in animals given 1 dose but in 2 out of 22 animals given 2 or 3 doses viraemia did occur for type 2. These animals had extensive CNS involvement but were not paralysed. Passage strains gave rise to viraemia, CNS lesions and paralysis much more frequently, particularly if other markers had also changed. Moreover, with the passage strains type 1 and 3 viraemia as well as type 2 occurred.

A. J. Beale

JUNGHERR, E. L., CABASSO, V. J., MOYER, A. W. & COX, H. R. **Proposed Method and Evaluation of the Monkey Neurovirulence Test for Attenuated Poliovirus Vaccine.** *J. Infect. Dis.* 1961, May-June, v. 108, No. 3, 247–61, 14 figs. [22 refs.]

This is a very valuable paper from Lederle Laboratories on the evaluation of the monkey neurovirulence of attenuated poliovirus strains. 4 points are stressed. First, that the neurovirulence test in monkeys is the only available test as other *in vitro* markers are unreliable. Second, the difference between attenuated and virulent strains is one of degree. Third, the pathological lesions and distribution are similar. Attenuated strains differ most clearly in lack of spread of lesions from site of placement in the CNS. Therefore spread is made the basis for the scoring system. Fourth, a uniform scoring system is essential for obtaining comparable results. The system is given in full.

A. J. Beale

AUSTIN, F. J. & MILES, J. A. R. **Observations on a Poliovirus Variant.** *J. Hygiene.* 1961, Mar., v. 59, No. 1, 57–63. [11 refs.]

The isolation of 4 strains of type 3 poliovirus during a survey in Dunedin, New Zealand, for adenoviruses is described; there was no clinical poliomyelitis in the district at the time. 1 was isolated from a child with conjunctivitis, 1 from the tonsil removed at tonsillectomy, 1 from a throat washing of a patient with mild upper respiratory illness and 1 from the faeces of a child with biliary obstruction. All the strains were closely related antigenically and, although clearly type 3 polioviruses, were slightly different antigenically from the Saukett strain. 25,000 TCD50 of 1 strain of this virus did not paralyse a monkey after intracerebral inoculation. The fact that the virus was isolated from a tonsillectomy specimen without producting paralysis is taken as additional evidence of attenuation. The authors speculate that the presence of this virus in the community may have been the cause of the absence of clinical poliomyelitis in the area as the result of an interference effect. *A. J. Beale*

LENNETTE, E. H., SCHMIDT, Nathalie J. & MAGOFFIN, R. L. **Observations on the Complement-Fixing Antibody Response to Poliovirus in Patients with Certain Coxsackie and ECHO Virus Infections.** *J. Immunology.* 1961, May, v. 86, No. 5, 552–60. [17 refs.]

By using heated poliovirus complement-fixing [CF] antigens, cross-reactions and heterologous responses within the poliovirus types and with other members of the enterovirus group have been demonstrated [this *Bulletin*, 1958, v. 33, 1024; 1959, v. 26, 27, 1302]. In this paper the results of poliovirus CF antibody titration in 579 patients with proved Coxsackie, ECHO, mumps, herpes simplex, WEE or SLE virus infection are reported. Only 26 rises occurred and 4 were definitely due to dual infection or to vaccination and 9 others may have been the result of dual infection. The remaining 13 were instances of heterologous rises and were all of low titre and, generally, of short duration. ECHO 9 infection was frequently (18%) associated with such a heterologous rise and other ECHO viruses, although represented less frequently, may also have given rise to heterologous poliovirus antibodies. The study underlines the value of untreated poliovirus CF antigens for specific serological diagnosis.

A. J. Beale

LEHMANN-GRUBE, F. **Comparative Susceptibility of Mammalian Cells in Culture to Prototype Enteroviruses.** *Arch. f.d. gesamte Virusforschung.* 1961, v. 11, No. 2, 276–83. [42 refs.]

"The cytopathogenicity of 51 enterovirus prototype strains on primary human amnion cell cultures has been determined. All 3 polioviruses, Coxsackie A-9, 11, 13, 15, 18, B-1 to B-5, ECHO 1 to 3, 5 to 13, 15, 19 to 21, and ECHO 24 caused consistent destruction of the cells. Coxsackie A-10 and A-14 which gave variable results with the original mouse material, could be adapted on passage. For comparison the same viruses were tested in 2 more cell systems of primate origin. Monkey kidney cells were destroyed by all poliovirus types, Coxsackie A-9, all Coxsackie B strains and ECHO 1 to 20, 22, 23, and 24. Coxsackie A-14, and A-16 as well as ECHO 21 could be passed with slight CPE, HeLa cells, on the other hand, were affected in a visible manner by poliovirus 1 to 3, Coxsackie A-11, 13, 15, 18, B-1 to B-5 and ECHO 1, 2, 3, 5 to 13, 15, 19, 20, 22, and 24.

"Comparative titrations were performed on primary human amnion and monkey kidney cell cultures. The two cell systems were equally susceptible to the polioviruses, to Coxsackie A-9, Coxsackie B-1, 3, 5, and 17, out of 24 ECHO viruses. The combined range of the two cell systems covers 39 out of 51 enterovirus prototype strains. Primary human amnion cells are a useful and economical substitute for monkey kidney cells in assaying the majority of cytopathogenic enteroviruses."

HALONEN, P. **Immunization with ECHO Viruses grown in Monkey Kidney Cell Culture. Specific and Non-Specific Responses of Mice, Guinea Pigs, and Rabbits.** *Ann. Med. Exper. et Biol. Fenniae.* Helsinki. 1961, v. 39, No. 1, 15–28, 8 figs.

This paper deals with the important practical problem of producing good specific enterovirus antisera. 3 viruses grown in monkey kidney cells were used as antigens, ECHO 4 and 14 being poor antigens and ECHO 6 a good antigen. Mice produced poor levels of antibody but guineapigs and rabbits produced good levels. The author found that 2 or 3 injections at 3–4 weeks' interval was a much better course than the more usually employed weekly doses. Freund adjuvant increased the antibody titres. Non-specific cross-reactions due to host-cell antibodies were observed. These antibodies were most pronounced when adjuvant was used although in these experiments virus with a

high host antigen content was used in the inoculum. In complement-fixation tests specific fixation paralleled neutralization but, especially when Freund's adjuvant had been used, host antigen often fixed complement to as high titre as virus antigen. *A. J. Beale*

VAINIO, T. **Studies on Murine Hepatitis Virus (MHV3) *in vitro*.** *Proc. Soc. Exper. Biol. & Med.* 1961, June, v. 107, No. 2, 326–31, 5 figs. [17 refs.]

"It has been shown that murine hepatitis virus (MHV3) can be propagated serially in liver explant cultures. Cultures were prepared from fetal C57B1/6 livers and cultivated on rat-tail reconstituted collagen. MHV3 virus caused cytopathic changes in such explants that are mainly in the epithelial outgrowth and macrophages. Virus has been carried for 22 passages and has been recovered by assaying the culture material in mice. Using this system, the sera from mice infected with the virus have been tested and high titers of CPE inhibitory activity were demonstrated."

BROWNSTEIN, Barbara & GRAHAM, A. F. **Interaction of Mengo Virus with L Cells.** *Virology.* New York. 1961, July, v. 14, No. 3, 303–11, 4 figs. [12 refs.]

CASALS, J. **Procedures for Identification of Arthropod-Borne Viruses.** *Bull. World Health Organization.* Geneva. 1961, v. 24, No. 6, 723–34. [14 refs.]

The first steps are to determine whether a virus isolated is in fact arthropod-borne: the final evidence depends on reproduction of the natural cycle of events but this is usually impracticable, therefore the following steps give presumptive diagnosis, (a) circumstances of isolation, (b) propagation by serial inoculation of blood-sucking arthropods, (c) sensitivity to ethyl ether and sodium desoxycholate, (d) elimination of other viruses sensitive to these agents by serology (*e.g.*, mouse encephalomyelitis, encephalomyocarditis, lymphocytic choriomeningitis, Newcastle disease, psittacosis, herpes viruses), (e) behaviour in experimental animals and tissue cultures, (f) filtration and centrifugation, (g) demonstration of serological relationship to known arthropod-borne viruses. In practice several of these steps may be omitted.

The next set of procedures are designed to find out if the virus belongs to one of the antigenic groups of arthropod-borne viruses—these groups are described in detail. This depends largely on the preparation of a haemagglutinin or complement-fixing antigen. In general such relationships in groups A and B are best demonstrated by the haemagglutinin-inhibition tests, in group C and in the Bunyamwera group by the complement-fixation test. The preparation of grouping sera by inoculation of animals serially with 2 viruses in the same group is discussed and evaluated.

The last step is to establish the identity of the virus with a known agent or to demonstrate that it is previously unknown. With viruses shown to fall into a certain group the problem is one of comparison with all known members of the group. With ungrouped viruses comparison must be made with all the ungrouped agents. At this stage an antiserum to the new agent is essential and tests and antisera of maximum specificity are required. The stage to which identification can be carried depends on availability of sera and antigens to all the known arthropod-borne viruses. This is an important paper as it is a vey valuable summary of available information and techniques.

C. E. Gordon Smith

PORTERFIELD, J. S. **Cross-Neutralization Studies with Group A Arthropod-Borne Viruses.** *Bull. World Health Organization.* Geneva. 1961, v. 24, No. 6, 735–41, 3 figs. (2 on 2 pls.) [19 refs.]

Chick embryo fibroblast monolayers were used [this *Bulletin*, 1960, v. 35, 715], 15 group A viruses were compared. A stock virus suspension was plaque titrated. 60-mm. plates were then infected with a dilution giving 2,000–5,000 plaques per dish and 4 fish-spine beads containing antisera were placed on each. The diameters of plaque inhibition were read usually on the 3rd day. Middelburg virus was distinct from all the others. Eastern equine encephalitis was indistinguishable from the strain 142 isolated in Czechoslovakia. Western equine encephalitis showed a one-way relationship with Sindbis. 3 strains of Semliki Forest virus appeared identical. Uruma and Mayaro could not be distinguished. Semliki Forest, Chikungunya, and O'nyong nyong viruses can be arranged in a gradient: the last was inhibited by rabbit antisera to all 3, Chikungunya by homologous and Semliki serum only and Semliki only by homologous serum. O'nyong nyong and Chikungunya are thus distinct but very closely related as serum collected 14 days after inoculation of a rabbit with Chikungunya neutralized both viruses equally. O'nyong nyong mouse serum failed to neutralize Chikungunya.

By using both mouse and rabbit antisera 6 viruses were compared: O'nyong nyong could be distinguished from all the others but mouse sera failed to show clear differences between Chikungunya, Vereeniging, and TH-35, nor between Mayaro and Uruma. When dilutions of O'nyong nyong, Chikungunya, Vereeniging and TH-35 antisera were tested against O'nyong nyong virus, the slopes of the regression lines of the radius ^{2} of inhibition on dilutions were almost identical.

C. E. Gordon Smith

EIRING, A. G. & SCHERER, W. F. **Appearance of Persistently Cytopathic Eastern and Western Encephalitis Viruses after "Blind" Passage in Cultures of L Mouse Fibroblasts.** *J. Immunology.* 1961, July, v. 87, No. 1, 96–105, 4 figs. [21 refs.]

"Eastern and Western encephalitis viruses (EE and WE) were successfully passed serially in cultures of L mouse fibroblasts in 11 of 16 and 7 of 8 attempts, respectively. 'Blind' passage of virus was often required for development of a persistently cytopathic effect since although mouse brain suspensions usually destroyed

cells initially, cytonecrosis was commonly not evident during second to fourth serial passages even though mouse infectious virus was produced. Thereafter, each virus became persistently cytopathic and readily propagated through 7–20 (EE) and 7–37 (WE) serial passages. These findings emphasize the potential value of blind passage of specimens in cell cultures during attemps to isolate viruses from natural sources.

"Selection of cytopathic virus from the original mouse brain suspension is considered the most probable explanation of these observations, although other possibilities are discussed. The occasional failure of EE virus to propagate and the common disappearance of cytopathic virus effect during second to fourth serial passages of either virus remain unexplained, they seemed unrelated to the serum (rabbit, human, chicken or sheep) used to maintain cells, to duration of the interval between virus inoculation and harvest, and to autointerference. The latter phenomenon did not explain adequately the absence of definitive cytonecrosis during blind passages of virus, since interference could not be regularly produced upon *simultaneous* inoculation of L cell cultures with infectious and heat- or ultraviolet-inactivated WE virus."

HARDY, F. M. & HEARN, H. J., Jr. **The Formation of Plaques by Two Strains of Venezuelan Equine Encephalomyelitis Virus.** *Amer. J. Hyg.* 1961, May, v. 73, No. 3, 258–62, 6 figs. on pl. [10 refs.]

A strain (VE-1) of Venezuelan encephalitis virus isolated from donkey brain after 13 chick embryo passages and a variant of it were used. The latter (CILV-8) came from a culture of L cells chronically infected for 8 months. They were grown in monolayers or agar suspensions of L cells and chick embryo cells. In both types of monolayer VE-1 produced plaques with smooth edges 3·0–4·5 mm. in diameter after 48 hours' incubation. The CILV-8 strain produced, in both types of monolayer, 0·5–1·0 mm. plaques with rough edges after 72 hours. VE-1 produced spheroid 3·0 mm. plaques in agar suspensions of both types of cell. The agar suspension method was 10 times more sensitive than the monolayer method for VE-1. CILV-8 has not produced plaques in agar suspensions. Strains recovered from plaques after 2 plaque transfers had unchanged mouse virulence and plaque morphology.

C. E. Gordon Smith

HEARN, H. J., Jr., BROWN, A. & HARDY, F. M. **Alteration of Virulence of Venezuelan Equine Encephalitis Virus after Passage in L Cell Cultures and in Mice.** *J. Infect. Dis.* 1961, Mar.–Apr., v. 108, No. 2, 237–42.

A strain passaged in chick embryos and inoculated in doses of 10^3–10^8 mouse intracerebral (IC) LD50 caused cytopathic changes (CP) in L cell cultures and a maximum virus titre in 1–4 days. 10 or 100 LD50 gave similar titres without CP. 1 CPD50 was found equivalent to 100 tissue culture (TC) ID50 = 100 mouse ICLD50. 1 passage in L cells altered the T/C (TCID50/CPD50) from 100/1 to 1/1. This change was not maintained on passage in other hosts. The ratio M/C (mouse ICLD50/CPD50) of the original virus inoculum was 100/1, after 1 passage of a high dose in L cells it was 10/1, after a low dose it was 1000/1. Further passage of the latter material in L cells, however, reduced the M/C to 10/1.

A strain isolated from a culture of L cells chronically infected for 3 months with the same original inoculum had an M/C of 1/1. Passage once in L cells caused the M/C to revert to 100/1. After 7–8 months of chronic infection, strains isolated had a stable M/C of 1/1 which was retained through 3 serial passages in L cells. These strains had, moreover, completely lost pathogenicity for mice by the intraperitoneal route, which the previous derivants had not. Between the 5th and 8th serial passages in L cells, however, the M/C became 1/100 then 1/1000 and intraperitoneal avirulence for mice was retained. Passage of the original inoculum intracerebrally in mice gave an M/C ratio of 1000/1 while intraperitoneal passage gave a ratio of 100/1—equal to the original inoculum. Venezuelan encephalitis virus is very variable in its ability to cause CP in L cells in relation to its pathogenicity for mice, and the host used for passage appears to influence the selection of particular variants [see also this *Bulletin*, 1959, v. 34, 1302; 1960, v. 35, 1061].

C. E. Gordon Smith

NIR, Y. D. & GOLDWASSER, R. **Biological Characteristics of some of the Arbor Viruses *in vivo* and *in vitro*. I. Thermal Inactivation in Cell-Culture Medium.** *Amer. J. Hyg.* 1961, May, v. 73, No. 3, 294–6, 1 fig. **II. Multiplication and Cytopathogenicity of West Nile Virus in Kidney Tissue Cultures from Various Animals compared with the Susceptibility of the Animals *in vivo*.** *Ibid.*, 297–302, 1 text fig. & 2 figs. on pl. [15 refs.]

I. Suspensions of infected suckling mouse brain were diluted 1:10 in Medium 199 with 5% inactivated calf serum and incubated at 37°C. At intervals samples were titrated intracerebrally in 4-week mice. Eastern encephalitis virus (AR 167) had an initial titre of 7·2 log which fell only slightly in the first 8 hours, but 2·5 log after 24 hours. Infectivity could be detected after 72 but not 80 hours. Western encephalitis virus (L2-34a) had an initial titre of 5·6 log which was maintained for 4 hours. After 24 hours it had fallen 2·5 log and no infectivity was detected after 80 hours. Sindbis virus (AR-399) lost infectivity rapidly none being detected after 36 hours. St. Louis encephalitis virus (strain 904) lost no titre in the first 8 hours but infectivity was rapidly lost thereafter, being undetectable at 48 hours. West Nile virus (human strain) retained its titre for 8 hours then steadily decreased until no infectivity was detected at 72 hours. Ntaya virus lost 2·5 log in the first 8 hours and none was detected at 24 hours. Bwamba virus lost 3·4 log in 24 hours and no infectivity was detected after 30 hours' incubation.

II. A strain of West Nile virus isolated from man in Israel was used in its 15th suckling mouse passage. In

suckling animals inoculated intracerebrally the LD50 was 7·6 log in mice and hamsters and 3·5 log in rats; intraperitoneally it was 6·8 log in mice and 6·1 log in hamsters; subcutaneously it was 7·1 log in mice and 5·5 log in hamsters; intramuscularly it was 6·6 log in mice and 7·2 log in hamsters; orally it was 1·2 log in mice and 3·5 log in hamsters. Cotton rats and guineapigs were insusceptible by all routes as were rats except for the intracerebral route. In 3-month-old animals, all the above species were insusceptible by the subcutaneous, intramuscular or oral routes, and rats, cotton rats and guineapigs by the intracerebral and intraperitoneal routes also. In mice the LD50 by intracerebral inoculation was 6·5 log but they were insusceptible by intraperitoneal inoculation. The LD50 in hamsters was 7·0 log by both these routes. The viruses were grown in cultures of trypsinized kidneys from sucklings of the same species. The virus multiplied in all with maximum titres of 6·6 log in hamster kidney, about 4 log in mouse kidney, over 3 log in guineapig kidney and over 2 log in rat and cotton rat kidney. Cytopathic changes were seen only in hamster kidney after 48–72 hours and in mouse kidney after 8–10 days.

C. E. Gordon Smith

Doherty, R. L., Carley, J. G., Mackerras, M. Josephine, Trevethan, Patricia & Marks, Elizabeth N. **Isolation of Murray Valley Encephalitis and other Viruses from Mosquitoes in North Queensland.** Reprinted from *Australian J. Sci.* 1961, Mar., v. 23, No. 9, 302–3.

Fowls sent to North Queensland in November 1958 as day-old chicks were tested for haemagglutinin-inhibiting antibody to Murray Valley virus in July 1959: of 51 exposed in 5 places 13 had developed antibody. Between July 1958 and July 1959 12 children [total examined not stated] in 4 places developed Murray Valley HI antibody. Between July 1959 and February 1960 8 children at 4 places developed neutralizing antibody to AMM 2354 virus [Malayan Group A virus, this *Bulletin*, 1960, v. 35, 679]. Of 11 fowls exposed as day-old chicks at Mitchell River from October 1959, none had antibody in February 1960 but 6 had Murray Valley HI antibody in March and 9 in April 1960.

Mosquitoes of 21 species biting man and horses were collected in March-April 1960 at Mitchell River. Pools of about 50 were inoculated into newborn mice. Virus strains were obtained from 34 of 187 pools: 27 from *Culex annulirostris*, 2 from *Aedes normanensis*, 2 from *Anopheles bancroftii*, 2 from *A. meraukensis* and 1 from *A. farauti*. HI tests showed that 17 were in group B. Complement-fixation tests on these showed that 2 from *C. annulirostris*, 2 from *Aedes normanensis* and 1 from *A. bancroftii* appeared to be identical with Murray Valley virus. 9 from *C. annulirostris* (prototype MRM 16) were antigenically related to Murray Valley but appeared to differ both from it and from Japanese encephalitis virus. 3 strains from *C. annulirostris* (prototype MRM 32) were antigenically distinct from Murray Valley, Japanese encephalitis and MRM 16 viruses: these strains grew to lower titres in baby mice, gave longer incubation periods than Murray Valley c Japanese encephalitis and did not cause lesions on th chorioallantoic membrane. Cross-neutralization test showed the identity of the MRM 32 strains and showe that they differed from dengue-1 and dengue-2. HI an neutralization tests have distinguished 4 distinct anti genic groups among the viruses not in group B.

C. E. Gordon Smith

Anderson, S. G. & Ada, G. L. **The Action of Phos pholipase A and Lipid Solvents on Murray Valle Encephalitis Virus.** *J. General Microbiol.* 196 July, v. 25, No. 3, 451–8. [13 refs.]

"Phospholipase A (100 μg./ml.) acting at 37° fo 30 min. decreased the infective titre of crude or purifie Murray Valley encephalitis (MVE) virus from 7 or log to less than 1 log. This inactivated virus yielded log of infective 'ribonucleic acid' ('RNA') when it wa treated with phenol. Ether, chloroform or butanol act ing at 0–4° each decreased the infectivity of crude o purified MVE virus. Again the inactivated virus yielde 'RNA' with an infective titre higher than the titre o the inactivated virus. Infective 'RNA' prepared fron crude MVE virus was not inactivated by ether, chloro form or butanol. High concentrations of phospholipas A preparations destroyed 'RNA,' possibly due to trace of contaminating ribonuclease. The results suggest tha intact viral phospholipid is necessary for infectivity o the virus particle."

Tamalet, J., Toga, M. & Vignoli, R. L'encéphalit verno-estivale russe expérimentale de la souris Systématique des lésions et étude anatomo-cliniqu des crises convulsives. [**Experimental Russia Spring-Summer Encephalitis in Mice**] *Ann. Ins Pasteur.* 1961, Aug., v. 101, No. 2, 259–69, 3 tex figs. & 4 figs. on 2 pls.

The English summary appended to the paper is a follows:—

"1. Fifty-three mice were inoculated intracerebrall or intraperitoneally with a strain of Russian spring summer encephalitis virus. In the course of the disease three animals had spontaneous attacks of epilepsy an 41 others audiogenic convulsions, induced by acousti stimuli.

"2. Anatomical study of the brain of eighteen intra peritoneally inoculated mice showed characteristi primitive viral encephalitis lesions (vascular congestion perivascular infiltration and glial nodules). These wer predominant on the inferior and superior brain stem The cortical substantia grisea and substantia alba wer generally undamaged.

"3. Relationships can be demonstrated betwee appearance of epileptic fits and localization of anatomi cal lesions. Mice showing neither spontaneous no provoked epileptic fits, had inflammatory lesions of th inferior brain stem. On the contrary, epileptic mic showed more diffuse inflammatory alterations, in th inferior and especially superior brain stem (thalamus

diencephalon). The interpretation of these facts is discussed."

WRÓBLEWSKA-MULARCZYKOWA, Zofia. Właściwiści hemaglutynacyjne izolowanych w Polsce szczepów wirusa kleszczowego zapalenia mózgu i ich budowa antygenowa badana za pomocą odczynu zahamowania hemaglutynacji. [**Haemagglutinating Properties of Tick-Borne Encephalitis Virus Strains isolated in Poland and their Antigenic Structure studied by Haemagglutination-Inhibition Test**] *Med. Dośw. i Mikrob.* Warsaw. 1961, v. 13, No. 3, 251–9, 1 fig. [19 refs.]

The English summary appended to the paper is as follows:—

"The hemagglutinating properties of 10 strains of tick-borne encephalitis virus isolated in Poland in 1953–57 were investigated. HA-antigens were prepared according to the method elaborated by Clarke and Casals. Goose red blood cells were used, various *p*H and temperatures were applied.

"All the Polish strains as well as the Moscow 256 strain showed hemagglutination of goose red blood cells. The optimal *p*H—6·4.

"The results of the cross-hemagglutination-inhibition tests with the corresponding rabbit immune sera as well as with the standard Moscow 256 and WEE (western equine encephalomyelitis virus) antisera indicate that the Polish strains fit into the group B of arbor viruses. Marked similarities were found among all strains tested as well as their close relationship to the standard strain Moscow 256. Some differences among the strains seem to be caused by the differences in the complexity of their antigenic structure."

ZHDANOV, V. M., PYRIKOVA, A. P. & FADEEVA, L. L. **Use of the Haemagglutination-Inhibition Test in the Study of Museum Strains of Neuroviruses.** *Bull. World Health Organization.* Geneva. 1961, v. 24, No. 6, 743–8. [17 refs.]

Haemagglutinins were prepared by centrifugation of *p*H 9·0 saline extracts of infected mouse brain. They were rendered non-infective by long storage at 4°C. or by addition of methylene blue and irradiation with light. Rabbit antisera were absorbed with goose cells and kaolin. For antigens of tick-borne encephalitis and diphasic meningo-encephalitis optimal titres were obtained at *p*H 6·8–7·0 at 4°C. In cross haemagglutinin inhibition tests there were no significant differences between tick-borne encephalitis and diphasic meningo-encephalitis strains: there was a marked cross-reaction with Kyasanur Forest disease and TP-21 [Langat] viruses but a minimal one with Japanese encephalitis. Some cross-reaction was found between western and eastern encephalitis viruses and 3 Czechoslovakian strains of eastern appeared to be identical with the American strain. *C. E. Gordon Smith*

NICOLAU, S. S., DRĂGĂNESCU, N. & CIEN TÎN HUAI. Cercetări experimentale asupra eficacităţii vaccinului antiencefalitic (encefalita de căpuşă) preparat cu ajutorul ionilor de argint. [**Experimental Investigations on the Efficiency of a Tick-Borne Encephalitis Vaccine Prepared by means of Silver Ions**] *Studii si Cercetări Inframicrobiol.* 1961, v. 12, No. 1, 9–17. French summmary.

A tick-borne encephalitis virus vaccine was prepared by the method of inactivation of the virus with silver nitrate described by the authors in earlier works [*Trop. Dis. Bull.*, 1959, v. 56, 1231; 1960, v. 57, 901].

A 10% suspension of mouse brain infected with the virus was prepared in 0·15% silver nitrate solution and kept at room temperature for varying periods up to 120 hours; the excess silver nitrate was then removed by centrifuging and resuspending the sediment in distilled water with subsequent addition of sodium chloride to produce isotonicity. By intracerebral inoculation of mice it was established that infectivity was lost after exposure to silver nitrate for 96 hours. In similar experiments in which the mixture was kept at 37°C. it was found that inactivation was complete after 44 hours. Mice vaccinated with 3 doses of the latter material withstood intracerebral challenge with 8·4 LD50 of live tick-borne encephalitis virus carried out 10 days after the last dose, and 4 of 5 mice survived challenge with 4 LD50 after vaccination with material which had been stored for 120 days. *D. J. Bauer*

WIEBENGA, N. H. **The Cultivation of Dengue-1 (Hawaiian) Virus in Tissue Culture. I. Carrier Culture of Human Skin Cells infected with Dengue-1 Virus.** *Amer. J. Hyg.* 1961, May, v. 73, No. 3, 350–64, 4 figs. [20 refs.]

——, with the technical assistance of I. E. FISHBURNE. **II. Cytopathogenic Virus subcultured from HuS 2806 Dengue-1 Carrier Culture.** *Ibid.*, 365–77, 5 figs.

[Abstracts of these papers appeared in the *Tropical Diseases Bulletin*, 1961, v. 58, 1234.]

BAYER, M. E. & NIELSEN, G. Zur Morphologie des Gelbfiebervirus. (Kurze Mitteilung.) [**Morphology of Yellow Fever Virus**] *Arch. f. d. gesamte Virusforschung.* 1961, v. 11, No. 2, 303–6, 3 figs.

[An abstract of this paper appeared in the *Tropical Diseases Bulletin*, 1961, v. 58, 1234.]

BROOKE WORTH, C., DE SOUSA, J. & WEINBREN, M. P. **Studies on the Life-History of *Aëdes (Skusea) pembaensis* (Theobald) (Diptera, Culicidae).** *Bull. Entom. Res.* 1961, June, v. 52, Pt. 2, 257–61.

Mosquitoes were collected coming to bite man and resting on vegetation, on the ground, and in crab holes around a mangrove-bordered tidal estuary near Lumbo

on the coast of northern Mozambique. *Aedes pembaensis* was found to breed by laying eggs on the proximal segments of the front legs of the crab, *Sesarma meinerti*. Eggs were restricted to crabs on areas which were flooded only at higher tides. *A. pembaensis* formed 61% of the mosquitoes taken in 1959 and 69% of those taken in 1960. Females were found to range inland freely for about 1 mile but were sparse 4 miles inland. 3 white volunteers were exposed to trapped mosquitoes—2 were apparently unpalatable and bites on them were painless and followed by no cutaneous reaction. The third was highly attractive and had painful bites and severe cutaneous reactions. 64 bloodmeals in *A. pembaensis* were identified as human. Of 25 in *A. albocephalus*, 24 were human and 1 apparently goat. From females of *A. pembaensis* 8 strains of an apparently new virus were isolated in 1959 and 4 in 1960. In addition, in 1960, 2 strains of Bunyamwera virus were isolated from them. In 1959 human sera collected in the area showed only a low-incidence antibody to the new virus (Lumbo virus). Of 30 sera collected in 1960 at least 4 had neutralizing antibody to Lumbo virus and 6 haemagglutinin-inhibiting antibody to Bunyamwera virus. No viruses were obtained from *A. albocephalus*. *C. E. Gordon Smith*

Oddo, F. G. & Sinatra, A. Studi sulla riproduzione del virus morbilloso in colture di tessuti *in vitro*. [**Studies on the Multiplication of Measles Virus in Tissue Culture *in vitro***] *Riv. Istituto Sieroterap. Ital.* 1961, Mar.–Apr., v. 36, No. 2, 65–89, 19 figs. [22 refs.] English summary.

The authors have studied the rate of multiplication of the Edmonston strain of measles virus in cultures of HeLa cells. On infection with a multiplicity of 0·12–0·40 TCD50 per cell the titre of intracellular virus began to rise after 16 hours and attained a maximum of 10^5 TCD50 per ml. after 48 hours; the titre of virus in the supernatant fluid followed a similar course at a slightly lower level. The percentage of infected cells as determined by treatment with fluorescent antibody reached a maximum of around 80% after 56 hours. In similar experiments with a multiplicity of 12·7 TCD50 per cell the maximum titres of virus were not much higher, but were attained 24 hours after infection. Syncytia could be found after the same period, and a cytopathic effect of the kind characteristic of measles virus could be observed in stained preparations.

D. J. Bauer

Shingu, M. & Nakagawa, Y. **Studies on the Measles Virus, the Isolation of Measles Virus on the HeLa Cells, and Immunogical and Morphological Properties of the Isolated Agents.** *Kurume Med. J.* 1960, v. 7, Nos. 2/3, 82–8, 4 figs. [14 refs.]

"In this communication a report is made on the measles virus.

"The results are summarized as follows:

"1. The three new strains of the measles agent were isolated from the blood of 7 measles patients in HeLa cells.

"2. The convalescent sera of such patients neutralize specifically the virus when tested by inoculation in HeLa cells or FL cells.

"3. Characteristic intranuclear inclusions, intracytoplasmic inclusions and multinucleated giant cells have been found in FL cells after infection with isolated agents.

"Three strains, named Noguchi strain, Takeshima strain and Kawahara strain, were identified as the agent of measles by immunological and morphological studies."

Toyoshima, K., Hata, S. & Miki, T. **Virological Studies on Measles Virus. IV. The Effect of Active and Inactivated Measles Virus on Cultured Cells.** *Biken's J.* Osaka. 1960, Dec., v. 3, No. 4, 281–91, 13 figs. (8 on 4 pls.) [16 refs.]

In a study of the course of formation of giant cells in cultures of FL cells infected with the Toyoshima strain of measles virus it was possible to observe fusion of cells 1 hour after infection both with active virus and with virus inactivated by ultraviolet irradiation. By the addition of specific antiserum at intervals after infection it was shown that exposure to live or irradiated virus for 90 minutes was sufficient to cause the maximum formation of giant cells. Over a certain range the number of giant cells formed bore a linear relationship to the logarithm of the amount of virus used for infection. Virus inactivated by heating did not cause the formation of giant cells. In cultures treated with trypsin and citric acid the number of nuclei thus isolated did not differ greatly from control values in cultures infected with irradiated virus, but was greatly reduced in preparations from cultures infected with live virus or with virus inactivated by heating.

D. J. Bauer

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Uhr, J. W. & Baumann, Joyce B. **Antibody Formation. I. The Suppression of Antibody Formation by Passively Administered Antibody.** *J. Exper. Med.* 1961, May 1, v. 113, No. 5, 935–57, 10 figs. [45 refs.] **II. The Specific Anamnestic Antibody Response.** *Ibid.*, 959–70, 2 figs. [22 refs.]

I. "The suppression of antibody formation by passively administered antibody is influenced by the dose and nature of the antigen, type of immunization procedure, ratio of antibody to antigen, species origin and characteristics of the antiserum used, as well as the species selected for immunization. In guineapigs, diphtheria antitoxin formation can be effectively suppressed by an intravenous injection of excess homologous or heterologous antitoxin as long as 5 days after toxoid immunization and after delayed-type hypersensitivity to toxoid has developed. Following the period of antibody

suppression which lasts 2 to 7 weeks, serum antibody can usually be demonstrated. It is proposed that this delayed immunization results from dissociation of antigen, since diphtheritic paralysis and death can be produced in guineapigs and rabbits by the intravenous injection of toxin-antitoxin precipitates formed in antitoxin excess. This syndrome is prevented by injection of excess horse antitoxin 1 hour after injection of the toxin-antitoxin complexes."

II. "Diphtheria toxoid-antitoxin precipitates formed in antitoxin excess can prepare guineapigs, rats, and rabbits for a secondary type of antitoxin response. Priming may occur without the development of detectable serum antibody. In rats, toxoid-antitoxin precipitates are more efficient than 'free' toxoid in priming, whereas in guineapigs, the magnitude of the anamnestic response varies with the precipitate employed. The possibility that priming is due to 'free' antigen released from the specific precipitate rather than the precipitate itself is discussed. The anamnestic antitoxin response can be inhibited by passive antitoxin, but less efficiently than primary antitoxin formation. Partial suppression of the secondary antitoxin response was accomplished by injection of excess horse antitoxin as long as 4 days after reimmunization with toxoid. The importance of these findings for the understanding of passive-active immunization in human beings is discussed."

AXELROD, A. E., HOPPER, Sarah & LONG, D. A. **Effects of Pyridoxine Deficiency upon Circulating Antibody Formation and Skin Hypersensitivity Reactions to Diphtheria Toxoid in Guinea Pigs.** *J. Nutrition.* 1961, May, v. 74, No. 1, 58–64, 3 figs. [20 refs.]

Pyridoxine deficiency has been shown to have a deleterious effect upon circulating antibody formation in the rat. In this paper the authors describe its effect in guineapigs: in young animals pyridoxine deficiency was produced by omitting pyridoxine from the diet, and in older guineapigs by administering the pyridoxine antagonist, deoxypyridoxine, to animals on a pyridoxine-deficient diet. In both groups of guineapigs there was depression of the circulating antibody response to the antigenic stimulus of diphtheria toxoid, as determined both by the tanned erythrocyte technique and by the Römer-Sames method for the estimation of diphtheria antitoxin. There was also depression of the early Arthus-type skin hypersensitivity reaction to diphtheria toxoid. These results, considered with other work, lead the authors to think that there is a firm rationale for the suggestion that vitamin deficiency states, induced by dietary means or the application of vitamin antagonists, may be utilized to ameliorate the undesirable sequelae of hypersensitivity reactions. *D. A. Cannon*

BARR, Mollie & FAIRLEY, G. H. **Circulating Antibodies in Reticuloses.** *Lancet.* 1961, June 17, 1305–10, 6 figs. [Numerous refs.]

Owing to the failure to distinguish between primary and secondary immunization the ability of patients with reticuloses to produce antibodies is variously described. In this work an immunizing course of two injections of one ml. of tetanus toxoid 6 weeks apart was given to patients suffering from different types of reticulosis who had no previous immunity ("primary immunisation") and to those who had been immunized in the past ("reimmunisation"). Samples of blood were taken at the time of each injection and two weeks after the second. The resulting antitetanus activity was assayed by mouse protection tests and in addition anti-A isoagglutinin titres were determined (by agglutination and haemolysis) since this substance is present in commercial toxoid.

Primary Immunization. 21 patients with malignant lymphocytic disease (chronic lymphatic leukaemia or lymphosarcoma), 21 with other reticuloses (*e.g.* Hodgkin's disease and myeloproliferative syndromes) and 10 controls were given an immunizing course. None showed any initial antitetanus titre. The titres, 2 weeks after the second injection, however, showed that the response of the patients with reticulosis was impaired: those with malignant lymphocytic disease were more affected than those with other forms of reticulosis. A good correlation was observed between abnormal (high or low) serum gamma globulin levels and inability to form antitoxin. No correlation was established between antitoxin formation and the duration, severity or extent of the reticulosis or with the age of the patient or therapy whether by cortisone, cytotoxic drugs or radiotherapy.

Re-immunization. 5 controls, 6 chronic lymphatic leukaemic patients and 9 with other reticuloses who were re-immunized all produced normal or only slightly impaired antitoxin levels in the second and third blood samples. Patients with chronic lymphatic leukaemia, however, gave very low titres and all of these were observed to have a low gamma globulin level. The authors consider that "in a population like this in which individuals have been immunised at different times the production of tetanus antitoxin is variable so that no definite conclusions can be drawn; but there is a suggestion that in chronic lymphatic leukemia antibody formation is impaired particularly in the presence of hypogammaglobulinaemia." The response to anti-A substance in these patients supported the findings with tetanus toxoid. *Stewart Fletcher*

SUSSDORF, D. H. **Rise of Antibody Titre in Cell-Free Spleen Preparations incubated *in vitro*.** *Nature.* 1961, Aug. 19, v. 191, 770–72, 2 figs.

FRIEDMAN, R. M., BUCKLER, C. E. & BARON, S. **The Effect of Aminoethylpteroylglutamic Acid on the Development of Skin Hypersensitivity and on Antibody Formation in Guinea Pigs.** *J. Exper. Med.* 1961, Aug. 1, v. 114, No. 2, 173–83, 1 fig. [26 refs.]

"1. In guineapigs, aminomethylpteroylglutamic acid (methotrexate) is capable of blocking the development of delayed skin hypersensitivity, the primary antibody response, and the specific febrile response to ovalbumin

and diphtheria toxoid. The primary antibody response is more easily inhibited than is the development of delayed skin hypersensitivity.

"2. The effect of methotrexate on immunologic responses depended upon the dose of methotrexate employed and the strength of the antigenic stimulus."

POTTER, Elizabeth V. & STOLLERMAN, G. H. **The Opsonization of Bentonite Particles by γ-Globulin.** *J. Immunology.* 1961, July, v. 87, No. 1, 110–18, 5 figs. [19 refs.]

VARGUES, R., GRENIER, B. & MORAUD, B. Dosage du pouvoir bactéricide des sérums par mesure de la respiration bactérienne. Application à l'étude des propriétés des sérums hypergammaglobuliniques. **[Assay of Bactericidal Activity of Sera by means of the Evaluation of Bacterial Respiration, Application to the Study of Hypergammaglobulin Sera]** *Ann. Inst. Pasteur.* 1961, July, v. 101, No. 1, 56–75, 5 figs. [16 refs.]

The English summary appended to the paper is as follows:—

"The bactericidal activity of sera is easily evaluated by the study of the action of these sera (heated or not) on the respiration of bacteria such as *E. coli.* Serum complement is absolutely necessary to inhibit the bacterial respiration: guinea-pig serum, which is deprived of any bactericidal activity, is a good source of complement. In presence of constant amounts of complement, there is a direct relationship between the activity of the sera and the amount of serum used. The optimal amount to inhibit in thirty minutes the respiration of one milliard *E. coli* is 0·05 to 1 ml. Heated sera (65°) are somewhat less active (in presence of complement excess) than fresh sera.

"One hundred and thirty hyper-γ-globulinic sera were studied. Significant decrease of the bactericidal activity was observed in marked dysproteinemia (macroglobulinemia, Kahler's disease) and in 20% of the cases of hepatic cirrhosis. In infectious hepatitis and in cases of common dysproteinemia in Africans the bactericidal activity is about the same in hyper-γ-globulinic and normal sera.

"There is no relationship between the decrease of bactericidal activity of certain hyper-γ-globulinic sera and the titer of γ-globulins or the titer of complement. This decrease is not due to the anti-complementary activity of γ-globulins."

CHAHAVA, O. V. **Research on the Effect of Sterilizing Doses of Co^{60} Gamma Rays on Antibacterial Sera.** *J. Hyg., Epidemiol., Microbiol. & Immunol.* Prague. 1960, v. 4, No. 2, 196–206, 2 figs. [19 refs.]

The object of the investigations described in this paper was to determine the possibility of sterilizing diagnostic immune sera by irradiation. The source of irradiation employed was an experimental cobalt bomb and the sterilizing dose was $1{\cdot}5 \times 10^6$ r at a dose-rate of 600 r per minute. The sera were obtained from rabbits immunized with different typhoid vaccines, made from strains of O-901, H-901, Ty2 and also the Vi containing strain of *Escherichia coli,* 5396/38. Antibody estimations included agglutination, haemagglutination, electrophoresis and electrophoresis-haemagglutination (for the determination of the antibody-localization in the sera). The sera were subjected to various forms of treatment before irradiation, and the treated sera, without irradiation, were compared with sera treated and irradiated in so far as the agglutinin and haemagglutinin levels were concerned.

The results may be summarized thus: all irradiated sera show loss of antibody. The order of the loss is Vi, O and H. That is to say Vi antibody is most sensitive to irradiation and only lyophilization protects against its effect. O antibodies are next in sensitivity, O agglutinins being more sensitive than O haemagglutinins. The addition of cysteinamine also appears to offer some protection against antibody loss through irradiation. The localization of antibodies in the serum protein fraction did not appear to be markedly changed by irradiation. The results of mouse protection tests indicated that the protective power of sera after gamma-ray treatment was not impaired. *H. J. Bensted*

BATTY, Irene & BULLEN, J. J. **The Permeability of the Sheep and Rabbit Intestinal Wall to Antitoxin Present in the Circulation.** *J. Path. & Bact.* 1961, Apr., v. 81, No. 2, 447–58, 6 figs. (3 on pl.) [16 refs.]

These authors have already shown [this *Bulletin,* 1957, v. 32, 378] that antibody protein is absorbed from the intestine into the circulation of adult mice and sheep. Expecting that such transmission can occur in either direction, depending on the relative concentration of antibody protein in the circulation and the intestine, they compared the titres of antitoxin in the serum and intestinal contents of rabbits and sheep injected with mixtures of untreated (crude) and pepsin-refined sera (*Staphylococcus* α-antitoxin, *Clostridium welchii* α-, β-, and ε-antitoxins) prepared in either sheep or horses. In some rabbits the gut contents were sampled after perfusion of the vascular system; in others, by catheterization through the stomach. The sheep were equipped with permanent fistulae into the rumen, duodenum and ileum.

A small but readily estimated proportion of the antitoxin, injected intravenously into normal rabbits and sheep, appeared in the intestinal contents. In sheep actively immunized against *Cl. welchii* type D, *Cl. welchii* ε-antitoxin was found in the duodenum and ileum in concentrations ranging from 0 to 0·8% of the serum concentration. The intestinal wall of the rabbit appeared to permit pepsin-refined antitoxin to pass through rather more readily than crude antitoxin, but was in the main unselective. (Comparison of the results of the experiments in which the gut samples were obtained by perfusion and catheterization showed that perfusion did not substantially alter the permeability of the intestinal wall to antitoxin.) The intestinal wall of

the sheep was only slightly more permeable to heterologous than to homologous antitoxin.

D. A. Cannon

COLOBERT, L.; KIRN, A.; SERVANT, P.; MONTAGNON, B. With the technical collaboration of R. GOUJEAUD. Les propriétés lytiques du sérum. III. Influence chez le singe de la vaccination antityphopara typhoïdique sur la teneur en obsistine [**Lytic Properties of Serum. III. Influence in Monkeys of Anti-Typho-Paratyphoid Vaccination on the Level of Obsistin**] [COLOBERT, KIRN, SERVANT & MONTAGNON]. *Ann. Inst. Pasteur.* 1961, May, v. 100, No. 5, 600–612, 6 figs. IV. Interférence du vaccin antipoliomyélitique sur la montée de l'obsistine provoquée chez le singe par la vaccination antityphoparatyphoïdique A at B [**Interference of Anti-Polio Vaccine on the Increase of Obsistin induced in Monkeys by Anti-Typhoid-Paratyphoid Vaccine**] [COLOBERT, KIRN, MONTAGNON & SERVANT]. *Ibid.*, 613–17, 2 figs.

The English summaries appended to the papers are as follows:—

III. "In monkeys obsistin takes part in the reaction of the organism to anti-*Salmonella* immunization. Its comportment is comparable, but not identical, to that of the agglutinating antibodies and that of the seroprotection capacity in experimental infection of mice.

"The titer of obsistin is maximal ten days after the third immunizing injection and reaches its initial level about three months and a half after this injection. At this moment, the ingestion of living *Salmonella* induces an increase of the serum level of obsistin; this increase is different in previously immunized and in non-immunized animals; in the former it is earlier and more intensive.

"The intraperitoneal injection of a massive amount of *Salmonella paratyphi* B does not induce any infection, but a strong increase of the serum level of obsistin, which is not parallel to that of the agglutinating antibodies."

IV. "The association of anti-polio vaccine with anti-typhoid-paratyphoid A and B vaccine disturbs the normal reactions to *Salmonella* in monkeys. The development of agglutinating antibodies and of the factors responsible for seroprotection in experimental infection of mice is decreased or slowed down, but the most striking change is observed in the evolution of the serum level of obsistin. In animals inoculated with a mixture of anti-polio vaccine + anti-typhoid-paratyphoid vaccine, the increase of the obsistin level is much less marked than in animals injected with anti-typhoid-paratyphoid vaccine alone."

KAUFMAN, L. & CHERRY, W. B. **Technical Factors affecting the Preparation of Fluorescent Antibody Reagents.** *J. Immunology.* 1961, July, v. 87, No. 1, 72–9, 1 fig.

CSEH, G. & SZABÓ, Ilona. **Studies on the Mechanism of Properdin-Zymosan Reaction. The Role of Polysaccharide Structure in combination with Properdin.** *Acta Microbiologica.* Budapest. 1961, v. 8, No. 2, 181–7, 2 figs. [24 refs.]

"(i) The properdin-reacting and protein-adsorbing capacity of the cell wall polysaccharide of *Saccharomyces cerevisiae*, zymosan and that of its components, glucan, mannan and glycogen have been investigated. Examinations were performed also with other polysaccharides and inorganic adsorbents.

"(ii) The combining capacity with properdin decreased in the following order: zymosan, mannan, glucan, cellulose, calcium phosphate gel and caolin.

"(iii) With solid phase-forming substances the interaction with properdin was connected with a definite degree of protein adsorption.

"(iv) Of the examined substances, the most specific combination with properdin was obtained with zymosan.

"(v) In the presence of Ca and Mg ions mannan inhibited the reaction between properdin and zymosan. No inhibitory action was exerted by glycogen.

"(vi) The reaction between properdin and glucan differed from that between properdin and zymosan in stability and ion requirement.

"(vii) It has been concluded that the high specificity of zymosan in the reaction with properdin is due to the structural properties of mannan."

KUBELKA, V. & RYBÁK, M. **Action of Tetracycline on the Level of Properdin.** [Correspondence.] *Nature.* 1961, Aug. 19, v. 191, 820–21, 2 figs.

MIYAZAKI, M. **On the Effect of Certain Heavy Metals upon the Complementary Action.** *Med. J. Shinshu Univ.* Matsumoto. 1959, Sept., v. 4, No. 3, 335–43. [11 refs.]

NICOL, T., MCKELVIE, P. & DRUCE, C. G.; NICOL, T., BILBEY, D. L. J. & DRUCE, C. G. **Phagocytic Activity of the Reticulo-Endothelial System.** *Nature.* 1961, Apr. 29, v. 190, 418–20. [12 refs.]

Active oestrogens having previously been reported to be strong stimulants of the phagocytic activity of the reticulo-endothelial system, the authors decided to try the effect of a non-oestrogenic substance, and chose BCG. Male white mice were given one intravenous dose of 0·05 ml. of an 8-day culture of BCG on Dubos medium. At weekly intervals the phagocytic activity was measured by the rate of disappearance of a known amount of specially prepared carbon from the circulating blood; blood counts were also made and at post-mortem the livers and spleens were weighed. Phagocytic activity was increased slightly after one week, more markedly after two weeks, and reached a peak at the end of the third week. This activity was accompanied by a corresponding enlargement of both liver and spleen, a slightly reduced red-cell count but no alteration in the white-cell count.

Having previously shown (*Nature*, 1958, v. 181, 1538) that diethylstilboestrol was capable of stimulating phagocytic activity, the authors tried tri-p-anisyl-chloroethylene, dienoestrol and stilboestrol diphosphate, administered subcutaneously or orally to male white mice. The phagocytic activity was measured as described above. All three substances proved to be active stimulants of phagocytic activity, but tri-p-anisylchloroethylene alone failed to cause any enlargement of the liver, thus differing from the other synthetic oestrogens, and suggesting [without any evidence in this paper] that it may be the oestrogen of choice for clinical use in stimulating the reticulo-endothelial system and thus increasing the body defence against infection.

D. A. Cannon

BLOCH, H. **The Motley Phagocytes.** *New England J. of Med.* 1961, May 4, v. 264, No. 18, 914–19, 1 fig. [21 refs.]

In this, the fourth annual J. Howard MUELLER Memorial Lecture given at Harvard Medical School, the author comes to the conclusion that there is still a lot to be learned about the "eating cells" of Metschnikoff. Phagocytes and their role in infection must be evaluated from at least 3 different angles. Sometimes they are scavengers, picking up and eliminating bacterial invaders; at other times they are cells in which obligative or facultative intracellular parasites find a suitable environment for survival and multiplication; and thirdly, they are biological units in which the antigenic components of the parasites initiate and maintain the formation of antibodies which may eventually become instrumental in protecting the host from the effects of the parasites themselves.

Dealing mainly with observations on phagocytosis in cultured cell systems, the author discusses avirulent organisms (readily phagocytosed), more virulent organisms (which are ingested, damage phagocytes, are released, and go on multiplying), and capsulated organisms (which, in the absence of antibodies, are not phagocytosed at all). He reminded his audience of his work [this *Bulletin*, 1950, v. 25, 72; 1956, v. 31, 745; 1960, v. 35, 1101] and that of ELBERG [*ibid.*, 1960, v. 35, 1148] on tubercle bacilli: acquired resistance in tuberculosis expresses itself in an increased ability of macrophages to suppress the multiplication of tubercle bacilli and this ability may be further enhanced by a serum factor of unknown nature that is required after vaccination or infection and is more or less specific.

As for tuberculin and delayed-type sensitivity, macrophages from normal guineapigs, sensitized with tuberculin (PPD) *in vitro* and injected into fresh guineapigs, produce a solid, long-lasting tuberculin sensitivity in the absence of circulating antibody. Although the old question whether allergy in tuberculosis is "good" or "bad" remains unanswered, it looks as though there are at last some experimental tools available with which to approach this problem. There is, however, much to be learned about the morphological and biochemical differences that could account for the significant functional variations in phagocytic cells—they are, indeed, a motley crowd. *D. A. Cannon*

JETER, W. S., MCKEE, A. P. & MASON, Rachel J. **Inhibition of Immune Phagocytosis of *Diplococcus pneumoniae* by Human Neutrophiles with Antibody against Complement.** *J. Immunology* 1961, Apr., v. 86, No. 4, 386–91, 3 figs.

WARD and ENDERS in 1933 [this *Bulletin*, 1933, v. 8, 514] demonstrated that *in vitro* phagocytosis of *Streptococcus pneumoniae* by human polymorphs in the presence of type-specific antipneumococcal serum proceeded when complement had been inactivated by heating the serum at 56°C. for 30 minutes. The aim of the authors was to check this finding by using a method other than heat for the inactivation of complement, namely, antibody against complement prepared in rabbits. They found that treatment of normal human blood with rabbit antibody against human complement completely blocked the phagocytosis by the polymorphs of *Str. pneumoniae* in the presence of its type-specific antibody. (Normal rabbit serum did not seem to impede the ingestion process.) The results were similar whether the incubation period of blood with organisms was as short as 15 minutes or as long as 4 hours. When fresh, untreated serum was added to cells from treated blood, there was no reversal of the inhibition of phagocytosis. Bactericidal studies indicated that antipneumococcal serum was capable of destroying the organisms even when the complement was inactivated by anticomplement and the phagocytes appeared to be inactive—a rather parodoxical finding, since there is no known way in which these organisms can be destroyed in such a system apart from phagocytosis. *D. A. Cannon*

HIRSCH, J. G. & CHURCH, Alice B. **Adrenal Steroids and Infection: the Effect of Cortisone Administration on Polymorphonuclear Leukocytic Functions and on Serum Opsonins and Bactericidins.** *J. Clin. Investigation.* 1961, May, v. 40, No. 5, 794–8. [15 refs.]

"Polymorphonuclear leukocytes collected from rabbits given large doses of cortisone exhibit normal capacity to engulf and kill certain staphylococci and enteric bacteria. The general morphology and degranulation response to phagocytosis of these cells are not detectably different from those of leukocytes from normal rabbits.

"Granulocytes from normal and from cortisone-treated rabbits contain similar amounts of the antimicrobial agents lysozyme, phagocytin and histone.

"Administration of large doses of cortisone to rabbits is not associated with significant changes in opsonic or bactericidal activities of the serum."

Miscellaneous

HEASMAN, M. A. **Accidental Poisoning in Children.** *Arch. Dis. in Childhood.* 1961, Aug., v. 36, No. 188, 390–93.

The problem of accidental poisoning in children in the United States and Canada has been defined and discussed in a number of published papers as, for example, FLEMMING, 1960, where the United States Secretary of Health stated that the estimate made in the United States was "600,000 non-fatal and 500 fatal poisonings of children annually" (*Pub. Health Rep.* Wash., 1960, v. 75, 91).

The information contained in the present paper shows the position in England and Wales and is based on the morbidity and mortality data available in the General Register Office.

Analysis was made of the data obtained in a proportion of cases treated at National Health Service hospitals and on cases treated in general practice.

In 1958 an enquiry was made to collect data relating to 10% of all discharges from N.H.S. hospitals (excluding mental hospitals). From these data 1,000 consecutive records of poisonings were extracted, and of these, 218 related to children under 15 years of age. The findings in the analysis of these poisonings in children are described with a number of clear tables which set out the age and sex distribution, nature of the poison and length of stay in hospital. A calculation made on this sample of 218 children leads to an estimate that the annual total of cases of poisoning in children treated as in-patients is about 4,000. The annual number of cases seen by general practitioners only is unknown "but can be very approximately estimated at 8,000." The average annual number of deaths of children under 15 attributed to accidental poisoning has been 44 in the period 1954–58.

In summary "Boys are more commonly affected than girls and the maximum incidence is between 1 and 3 years of age. The commonest poisons leading to admission to hospital are aspirins, paraffin and turpentine. The commonest single cause of death is aspirin and salicylates." *M. E. Delafield*

DAMON, A. **Constitution and Smoking.** *Science.* 1961, Aug. 4, v. 134, 339–41. [14 refs.]

"Among 167 adult male factory workers of Neapolitan parentage but of American birth or upbringing, the lean men smoked significantly more than the fat ones. Smoking was positively correlated with serum cholesterol but was not associated with morphological masculinity, blood pressure, diet, or consumption of alcohol."

Book Reviews

WORLD HEALTH ORGANIZATION. PUB. HEALTH PAPERS No. 7. **Basic Nursing Education Programmes. A Guide to their Planning** [LYMAN, Katharine]. 81 pp., 2 figs. [Numerous refs.] 1961. Geneva: Palais des Nations. [Sales agent for U.K., H.M. Stationery Office.] [5s.; $1.00; Sw.fr. 3.–.]

This book is the final draft of a guide first prepared in 1957. The contents of the original guide were discussed by a special group of nurses with international experience, by members of the Expert Advisory Panel on Nursing, and by WHO staff members in various parts of the world. A second draft was completed in 1958 and sent to different parts of the world to be tried out in practice. This final draft now incorporates the various suggestions that were made in the light of this experience. It is, therefore, a distillate of much international discussion and experience.

The Guide is divided into two parts. The first, presented mainly in the form of questions, details the general and specific information that is required before planning for nursing education can begin. Attention is drawn to such general factors as geography, topography and climate of the country or area, to the composition of population, cultural patterns with particular reference to family ties and customs regarding illness and treatment, methods of transport, local language and dialect, system of government, economic resources and the general level of education. The next stage is consideration of the health needs and health services in the community, and the precise formulation of the functions that the nurses are to perform within the realities of conditions in the present and reasonably near future. Information should be obtained about the general pattern of nursing education and about the existing schools of nursing.

The second part of the Guide suggests steps for developing a nursing education programme. National planning as well as planning for an individual school are considered. The Guide also includes a list of recommended books on the subject. It concludes by putting forward a "golden rule" for planners, it is "that the plan must be made to fit the local situation and that all who will have a part in carrying out the plan should have a share in making it".

This Guide should be of value throughout the world. Few nursing schools, even in those countries which have had organized and supervised training for nurses for very many years, fulfil all the aims or have all the services suggested. How many nursing schools in Britain have an adequate library under the direction of a librarian? How many give each sister-tutor an office of her own and in how many is a system of health counselling readily available for the student nurses?

M. D. Warren

SIMON, Harold J. [M.D., Ph.D.] **Attenuated Infection. The Germ Theory in Contemporary Perspective.** Forewords by René J. DUBOS, Ph.D. & Walsh MCDERMOTT, M.D. pp. xvi + 349, 1960. Philadelphia. Montreal. J. B. Lippincott Co. [80s.]

This book is a perceptive and well documented account of attenuated infection in the plant and animal kingdoms. The author sets out a modern concept of the host-parasite relationship to show that attenuated infection is by no means a special and rare case. It is rather the common outcome of host-parasite interaction: overt disease is to be regarded as a relatively uncommon end-result.

Having first defined his terms, the author relates his work to the basic classical concepts of infectious disease. He discusses the occurrence and recognition of attenuated infection in nature and demonstrates with well chosen examples the many interplaying influences which govern the course of infection. The more important host factors are genetic constitution and physiological well-being: the effects of nutritional change, radiation, temperature variation, hormones, and age are among the many factors fully discussed. Evidence that host organ factors may be of local importance for attenuation in certain sites is reviewed. Changes in the parasite can also upset the equilibrium of the attenuated infection and examples such as infection with the influenza virus, infection with *Borrelia recurrentis*, and trypanosomiasis are mentioned in this context.

Synergic interactions between infectious agents may upset the attenuated state due to one alone. Antagonistic reactions between viruses are also known, which so suppress the activity of an infecting organism that the infection is attenuated. The advantages and disadvantages of attenuated infection to both host and parasite are dealt with in some detail.

Viral infections of mammals, insects, and plants exemplifying the three subdivisions of attenuated infection (carrier state, persistence and latency) have been brought to light in tissue culture. The author devotes a complete section to this technique with which many hypotheses regarding viral diseases can be tested under controlled conditions. The use of tissue culture for the study of metabolic aspects of the host-parasite relationship has clearly much unexplored potential.

The significance of the altered and filtrable forms of bacteria isolated sporadically from tissue culture is rationally discussed.

Attenuated infection in man himself is given almost a third of the entire book and a rather more discursive treatment. Cultural, economic, psychological and emotional factors are additional influences affecting this particular host in his relationships with parasites. The sections dealing with the clinical recognition of attenuated infection and with those clinical practices which may activate the attenuated state to progressive infection, are particularly interesting to the medical mind: they indicate the value of what the author terms "a biologically oriented approach".

The author has drawn his material from widespread and varied sources. He should have an equally wide interested readership among scientific biologists.

J. M. Stark

KLEMPARSKAYA, N. N., ALEKSEYEVA, O. G., PETROV R. V. & SOSOVA, V. F. **Problems of Infection, Immunity and Allergy in Acute Radiation Diseases.** viii + 165 pp., 34 figs. [Translated from Russian.] 1961. London. New York. Paris Oxford: Pergammon Press Ltd., Headington Hill Hall. [50s.]

The authors of this remarkable book are themselves experimentalists; this gives additional weight to their explanations of many very varied observations.

Judging by the list of references to the literature on page 147, it seems that the Russians certainly come first in this difficult field in radiology for out of 326 references they figure in 179. There are 5 chapters. The first concerns "changes in Automicroflora and Microinvasion in the Irradiated Organism" (pp. 1–14). The experimental data derived from whole-body irradiation show:—(1) The irradiated organism becomes more susceptible to infections induced not only by pathogenic micro-organisms but also by bacteria of the automicroflora; (2) Decreased resistance to infectious agents occurs with the development of radiation sickness and is especially pronounced during the period of its acute course; (3) Infection complications may be a direct cause of death in radiation sickness. Readers may be reminded that this conclusion was also reached concerning the mode of death of many of the Hiroshima victims.

Chapter II is on the "State of Natural Immunity in an Irradiated Organism". The balance is upset by whole-body irradiation. In Table V are listed a number of immunobiological reactions in dogs after a dose of 600 r. These data are not only interesting in themselves but also because they show the individuality of each of the dogs in their striking differences of reaction to the same dose. For example in animals S, M and D we have:—

		Before	*After Xrays*
Percentage of phagocytosis	S	14·6	2
	M	10	48
	D	15.8	9
Microflora of skin (no. of bacteria per 12cm.² of skin)	S	164	400
	M	403	510
	D	115	30
Percentage of cultures breaking down mannite	S	7·8	25
	M	1	15
	D	17·3	44

With variations like this, generalizations must be very guarded and the authors realize this.

Chapters III (pp. 33–65) and IV (pp. 66–104) deal with induced immunization, the state of passive immunity in irradiated animals and some of the pecularities in allergic reactivity.

Chapter V deals with "Infectious diseases in Radiation Sickness" (pp. 105–143).

A few pages by way of Conclusions (pp. 144–146) could well be read before beginning on the main text. This book is a valuable forerunner and should command a wide circle of readers.

S. Russ

Printed in Great Britain by The Eastern Press, Ltd., London and Reading